The environmental impacts of land based abalone aquaculture

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Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

University of Tasmania

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Declaration of originality

This thesis contains no material which has been accepted for a degree or diploma by this university or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis.

The work conducted in this thesis is solely the work of the candidate and there has been no assistance given in any of the experimental work except where stated in the Acknowledgements. The industry partner Abalone Farms Australia (AFA) and its staff provided advice about the farm operations only.

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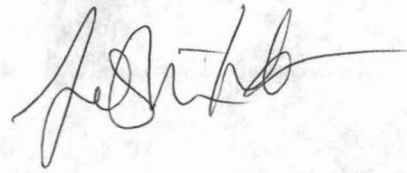

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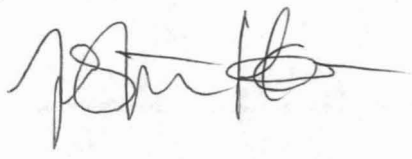

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Presentations to learned societies

Ho, J.D., Edwards S., Thompson, P.A. (2004) Towards understanding the environmental performance of land based abalone farming. Australasian Aquaculture, Sydney Convention Centre, Darling Harbour, Sydney, Australia, September 27-29th

Ho, J.D., Edwards S., Thompson, P.A. (2004) Towards understanding the environmental performance of land based abalone farming. 11th Abalone Aquaculture Workshop, Hobart Convention Centre, Hobart, Tasmania, Australia, 29-30 July 2004.

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The work conducted for this thesis was funded by Ausindustry and Abalone Farms Australia (AFA) in Bicheno, East coast Tasmania, Australia. An agreement between the investigators of this project, the industry partner (AFA) and the University of Tasmania exists whereby no material from this project may be published for a period of two years from the date of this thesis submission. The investigators of this project have negotiated with the industry partner who has agreed limited information may be published. These papers are currently under review. The subsequent chapters of this thesis have been written with the intention to submit for publication after 30th of July 2007.

Journal articles arising from the work described in this thesis.

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Ho, J.D., Edwards, S., Thompson, P.A. Characterisation of the variation in diurnal nutrient loads exported from a land based abalone farm: How time of day for sampling affects measured nutrient load. To be submitted to *Aquaculture.*

Ho, J.D., Edwards S., Thompson, P.A. Characterisation of the effluent from a land based abalone aquaculture facility and the prediction of effluent nutrient loads exported. To be submitted to *Aquaculture Engineering.*

Ho, J.D., Edwards S., Thompson, P.A. (2005) Environmental Impact Study of a land based abalone farm in Bicheno Tasmania, Australia. Marine Pollution Bulletin

Ho, J.D., Edwards S., Thompson, P.A. (2005) Reduction of effluent nitrogen from land based abalone farms by seaweed raft systems. To be submitted to *Aquaculture Engineering.*

Ho, J.D., Edwards S., Thompson, P.A. (2005) Results from the environmental monitoring of the Tasmanian land based abalone aquaculture industry. To be submitted to *Aquaculture.*

Ho, J.D., Edwards S., Thompson, P.A. (2005) Management of land based abalone farming in Tasmania. To be submitted to *Aquaculture.*

Abbreviations

A+A = Adam and Amos (formulated feed manufacturers)

AFA = Abalone Farms Australia (Industry Partner)

SSDs = Solids Separation Devices

DO = Dissolved Oxygen

TSS = Total Suspended Solids

BOM = Bureau of Meteorology

BOD = Biochemical Oxygen Demand

POM = Particulate Organic Matter

ml = millilitre

mls = millilitres

L = litre

^{o}C = centigrade

min = minute

mins = minutes

kg = kilogram

µm = micro moles

µM = micro molar

N = Nitrogen

P = Phosphate

Abstract

This study of land based abalone farming was conducted to investigate the industry's environmental impact and long-term sustainability. There is a paucity of information surrounding the environmental impacts of land based abalone farming despite the increase in the growth of the industry. Information which forms the basis of abalone farm environmental management and monitoring was developed as part of this project. This information is likely to provide a great deal of perspective to all existing and planned Australian abalone farms.

Three preliminary experiments were conducted to determine how nutrients were being produced within the abalone farm and how they were varying on a diurnal scale. Production of nutrients is clearly related to the formulated feed, however two possible means of nutrient production were deemed likely. Firstly from the formulated feed leaching or secondly from the degradation of the waste produced on farm. Finally a experiment was conducted to determine the pattern in nutrients exiting the abalone farm's effluent pipe.

The nutrient stability of a commercially available formulated feed was assessed. This is of importance as on most Australian abalone farms there is usually a delay between the time the formulated feed enters the abalone culture tanks, and the time of consumption by the abalone. This leads us to believe that there may be nutrients from the formulated feed. The formulated feed leaching appears to be significant in terms of phosphorus (30% of total P content), whilst it appears to be relatively insignificant in

terms of all other measured components. This research indicates that there may be a need for further research into the binding of phosphorus in Adams and Amos formulated feed.

An examination of the tank waste was conducted to examine if the waste within the abalone culture tanks was a major source of dissolved nutrients that are produced as the waste degrades. Results show that approximately 50% of the C,N,P and organics of the formulated feed was collected in the abalone culture tank waste. Further the settlement pond waste contained approximately 30% of the C,N,P and organics of the formulated feed. This equates to approximately 40- 50% of the abalone tank waste nutrients (i.e. particulate waste) being degraded and remineralised as dissolved nutrients.

The temporal variation in nutrient export for a single farm was characterised on a diurnal basis. Maximum concentrations of ammonium and phosphate were recorded at approximately sunrise at the farm outflow on the day of sampling. Therefore the time of day that water sampling takes place is important in the accurate assessment of environmental impact and may explain some of the variability in current monitoring program data sets.

Results indicate that of the nutrients measured from a 20 tonne abalone farm, dissolved nitrogen and phosphorus were predominantly exported with exports of, up to, 1000g and 280g per day respectively. The source of these nutrients is the formulated feed. Daily feed rate can predict dissolved nitrogen and phosphate loads exported from the farm ($P<0.05$, $r^2 = 0.98$ and 0.42 for N and P, respectively). The relationship between daily feed rate and nutrient export was found to hold at three abalone farms around Tasmania ($P<0.05$, $r^2 = 0.71$ for N and $r^2 = 0.62$ for P). These relationships can assist in the environmental management of the abalone industry and provide a consistent basis to

judge the impact of abalone farming relative to other sources of nutrient inputs into our coastal environment.

This study showed a 10-50 fold increase in biomass of nutrient scavenging seaweeds in the intertidal region in the vicinity of the abalone farm's discharge point. This was relative to both control sites and the discharge site prior to the commencement of the effluent discharge. Despite this the farm associated seaweed proliferation is unlikely to occur beyond 50 metres from point of discharge (i.e. at the end-of-pipe). Grazing and particulate feeding intertidal faunal communities were highly variable in time and space making it impossible to detect any effects of the farm's discharge. In the subtidal zone, there was no evidence of an impact on the macroalgal canopy assemblages within 50m of the end-of-pipe.

The importance of solid separation devices was also highlighted in this study. Output of particulates from farms without Solid Separation Devices (SSD's) is likely to be significantly greater than farms with SSD's (i.e. approximately 30-50% of feed input is lost as particulate waste). Relative to farm inflow waters, Abalone Farms Australia (AFA) did not increase particulate loads into the marine environment (measured by weight); however, even with farms with SSD's, the composition of the particulates in the discharge is likely to change. For example, AFA the discharged particulates had a slightly greater (i.e. 3% greater) organic content relative to intake particulates.

This study also showed that effluent nitrogen loads may be reduced by an average of 34% by a novel seaweed raft system held within the tanks. The seaweed raft system not only provided shade but most importantly a nutritious source of supplemental feed for the abalone.

The present study intensively examined a single farm, and extensively examined a number of farms to gain a perspective on the environmental impact of the Tasmanian abalone industry relative to other industries that discharge nutrients into the marine environment. The study found that the entire Tasmanian abalone industry as at 2005 was likely to have little more impact than a small town of 600 people's sewage treatment plant (based on total nitrogen discharge). Additionally detailed characterization of abalone farm effluent and the subsequent environmental impacts of that effluent was determined to be negligible for AFA at current production rates (i.e. 20 tonnes of abalone biomass).

Chapter 1: General Introduction

1.1 General Introduction

The growth in world aquaculture production is likely a result of a worldwide increase in demand for seafood, which is coupled with the stagnant and possibly declining catch from the world's wild fisheries (FAO, 1996). Aquaculture is a fast growing sector within Australia's primary industries expanding at a rate of around 20% per annum (CSIRO, 1999). Within this emerging sector, abalone aquaculture has attracted a great deal of attention given the high value of many Australian abalone species and also the declining catches in many of the worlds abalone fisheries (Department of Fisheries Western Australia, 2001). While the Australian abalone aquaculture industry is in its infancy at present, there is a great deal of potential for the expansion of the industry due to Australia's pristine water quality, close proximity to Asia, and acceptance of Australian species in the Asian markets (Love and Langenkamp, 2003). The expansion of any industry would be unwise without knowing the impacts of that industry on its environment. Long-term environmental sustainability is a key area of focus for the Australian aquaculture industry as lessons from other aquaculture industries around the world have shown us that environmental integrity is of great importance to the sustainable production of aquaculture species. Many industries worldwide have suffered production losses through diseases and poor environmental integrity (Buschmann et al., 1996; NACA/ACIAR, 2002; Páez-Osuna, 2001; Pro-Med News, 2003) and this highlights the importance of environmental health and sustainable practices for all users of aquatic resources. Of particular relevance to this issue are the recent abalone disease outbreaks in China and Taiwan (Nie and Wang, 2004; Pro-Med News, 2003; Zhang et al.,

2004), where significant mortalities have occurred and caused many farms in China to be economically unviable (Zhang et al., 2003). While the direct cause of this disease outbreak is not known and difficult to pinpoint, environmental factors and animal health issues remain central to most disease outbreaks (Subasinghe, 2005). Abalone health surveillance monitoring programs and abalone health have been researched and published in many countries around the world (Antonio et al., 2000; Lleonart et al., 2003; Mouton, 2003; Simon et al., 2004); however, there is no published research into the environmental impacts of abalone farming.

This lack of environmental information causes regulation problems due to the increasing competition for marine resources in Australian coastal waters (Reichelt and McEwan, 1999) which is coupled with a lack of information surrounding the relative environmental impacts of its users. This competition for resources combined with the precautionary principle, which is applied where there is a lack of environmental information, has caused a great deal of regulatory 'red tape' for many potential users of the resource. As aquaculture is a developing industry in Australia and is often subject to the precautionary principle, presently stringent environmental guidelines and restrictions face most aquaculture operations (Crawford, 2003a; Jackson et al., 2003b) relative to other industries. The abalone aquaculture industry has been subject to pressure by regulatory authorities to explore its interactions with the environment as no information exists regarding its affect on the marine environment. This is coupled with the fact that it is a point source discharge that can be easily monitored. The environmental pressure faced by abalone farmers is further fuelled by the environmental opposition of communities towards other aquaculture sectors such as salmon farming; which have

received recent media attention over the localised sediment impacts and sustainability of fishmeal usage (Crawford et al., 2001; Fairgrieve and Rust, 2003; Hannesson, 2003; Janowicz and Ross, 2001; McDaniels et al., 2005). Therefore abalone farming, and many other aquaculture industries, have tended to be placed in the same category as salmon farming along with their environmental effects, despite the fact that no research has taken place. Clearly studies which provide perspective as to the relative environmental impacts of different industries are of great importance as they provide coastal managers with tools for deciding the appropriate use of resources.

Abalone farming in Australia is largely land based farming (Hone and Maguire, 1996) where water is pumped from the ocean into culture vessels and then returned to the ocean usually by a single point source. Generally on a daily basis, formulated feed containing nutrients capable of supporting abalone growth are fed into the culture tanks, consumed and excreted. It is these processes of feeding the abalone which causes potential for environmental impact as it represents a large source of nutrient input into the farm and is an increasingly important aspect of aquaculture management.

Nutrients are commonly produced within most aquaculture facilities from a series of losses associated with formulated feed after it is fed, consumed, excreted and remineralised into the water column (by the breakdown by microbial or higher chemoheterotrophs). Abalone aquaculture is no exception, and further abalone are nocturnal and messy grazers that often take hours to locate, consume and then digest their feed (Shepherd, 1973; Shepherd, 1975).

Firstly on abalone farms there is the direct leaching from the feed which commences as soon as the feed in placed into the water. The amount of leaching is

determined by factors such as the binder type, diet particle size, water temperature and the physical movement of the diet once in the water (Findlay and Watling, 1994; Gowen et al., 1994; Myers and Zein-Eldin, 1975). In addition further leaching may occur in association with the physical breakdown of the feed by the abalone; which rasp their feed before ingestion (Shipton et al., 2002).

After ingestion a relatively small amount of the feed nutrients may be excreted as dissolved nutrients while the majority of the nutrients contained within the farm are likely to be bound as particulate waste (Jackson et al., 2003a; Krom and Neori, 1989; Maguire, 1998). Of this waste a portion may be remineralised into the water column (Barkai and Griffiths, 1987) while the other portion may settle to the tank floor and further be flushed from the tanks. This particulate waste may be retained within a solid separation device if one is present or simply discharged back into the ocean. It is also possible that if there is poor efficiency of the Solid Separation Device (SSD) that also some of the particulate waste may be returned to the ocean. Yet with the appropriate SSD design only dissolved nutrients should be returned.

Finally further losses of nutrients may occur through the degradation of the faeces which has been shown to be a major producer of nutrients within some aquaculture systems (Burford and Longmore, 2001; Burford et al., 2002). Faeces leach nutrients into the water column (Burford and Williams, 2001; Chen et al., 2003) and, if a solids sedimentation device is present, the majority of the particulate waste may be retained on site and broken down over time with nutrients remineralised into the water column (Hargreaves, 1998). Similar evidence of nutrient production has been shown in studies below salmon cages where fluxes of nutrients (particularly ammonium) from the labile

organic sediments to the water column commonly occur (Christensen et al., 1999; Hargrave et al., 1993).

In addition to the need to characterise the farming processes influencing any potential environmental impact there is the need to characterise the nature of the environmental impact on the coastal waters surrounding the abalone farm. Such a study will allow the biological significance of the abalone farm activities to be quantified and further assist in the assessment of whether a proposed farm is suitable for a specific location. The detection and quantification of environmental impacts is often a difficult task (Crowe et al., 2000; Kingsford, 1998; Underwood, 1991; Underwood, 1992; Underwood and Chapman, 2003) which employs the use of numerous control sites (Stewart-Oaten et al., 1986; Underwood, 1991; Underwood, 1992) in an attempt to document the impact relative to the natural variation of the area. Any changes outside of the trends exhibited at the control site(s) are suggested to be caused by the activities of the abalone farm. From an abalone farm there are two main sources of nutrient discharge, particulate and dissolved nutrients, both of which are likely to have very different environmental effects. Generally particulates have a localised effect around the receiving waters where accumulation of particulates can cause increased BOD (Islam et al., 2004; Teichert-Coddington et al., 1999), smothering of marine life (Loch et al., 1996), decreased macrofauna abundance and diversity (Brooks and Mahnken, 2003) and require processing by micro-organisms before nutrients can be remineralised and assimilated into the environment (Boyd, 1992; Hargreaves, 1998). Dissolved nutrients on the other hand are readily assimilated into the marine environment and relative to particulates may be transported greater distances away from the discharge point (Islam, 2005). Commonly

sources of nitrogen are limited in marine waters (Day et al., 1989) and the discharge of dissolved nitrogen and phosphate may lead to eutrophication (Bergheim and Brinker, 2003; Crawford, 2003a).

Following intensive investigations on waste characterisation and environmental impacts at a single abalone farm this study extends to an assessment of how representative this single farm is of the larger industry. This may be achieved through environmental monitoring programs and the current abalone environmental monitoring programs which exist around Australia fail to achieve this. Of the four states currently culturing abalone in Australia (Western Australia, South Australia, Victoria and Tasmania) there are only government regulated monitoring programs in two states, South Australia and Victoria which have been in operation for approximately 1 and 3 years and are compulsory in each state. The monitoring program in Victoria monitors basic water quality (nutrients and Total Suspended Solids (TSS)), yet no additional farm information is required. There is also no protocol for samples collection and farmers must take and submit the samples to a laboratory themselves. In South Australia there is a much more comprehensive monitoring program which requires information such as abalone biomass production, water usage, feed usage, effluent salinity, effluent nutrients, effluent TSS, disease reporting as well as chemical usage reporting. South Australia the farmers are required to take their own samples and submit them to a laboratory. Despite the collection of this information there are many limitations to the conclusions which may be made from the monitoring programs. For example, even the most vague of total nutrient loads is not possible to be calculated in the Victorian monitoring program as total water volumes has not been recorded (i.e. Total Load = concentration x volume). In both

programs, even if the loads can be calculated, are they valid? Unless there is a protocol for the collection of samples one cannot ensure the validity of the results as farmers have little scientific background. For example, does a farmer know about contamination sources of water samples (i.e. ammonium contamination), or do they know of the appropriate storage of samples (i.e. should be stored in the dark and kept cool). Similarly if a farmer decides to take sample for the monitoring program at 5pm during one sampling period and 8am at the next sampling period. How can we be sure that the difference in results is not an artefact of the difference in sampling times. This information is required for a coherent monitoring program. Currently there is no means of comparison for intra or inter-farm environmental impacts and nutrient exports from abalone farms. Hence if the factors influencing nutrient loads and environmental impacts can be characterised spatially and temporally (intra-farm variability); and if the results of a single study can be related to numerous farms, then this provides a basis for industry wide assessment protocols and more meaningful (comparative) and effective monitoring of abalone farms.

Finally once a perspective of the industry is gained and means of monitoring established, techniques into further reducing the environmental impacts of abalone farms can be made. This is important as all marine resource users have a social responsibility to conduct their business in a manner which is sustainable and reduces environmental impacts (Ecologically Sustainable Development Steering Committee1992). Research into the use of seaweed biofilters within aquaculture systems (Boarder and Shpigel, 2001; Neori, 1996; Neori et al., 2004; Neori et al., 1991; Neori et al., 1996; Neori et al., 2003; Neori et al., 1998; Neori et al., 2000) is one such technique to reduce environmental

impacts and it has been shown that abalone can be sustainably produced in co culture with seaweeds and other finfish and mollusc species with a minimum amount of waste. However these experimental systems are likely to require expertise in the culture of a number of different culture species and farming systems. Of particular interest to the investigators of this project was the co culture of seaweed and abalone. If a system were to be developed which could minimise the need for separate infrastructure for the culture of the seaweed and abalone within the same tank system, the low cost and potential benefits should be appealing to other abalone farming operations and potentially reduce nutrient export. Consequently low cost seaweed raft systems were developed and tested for their capacity to reduce effluent nutrients within the AFA farming system.

The study site was chosen through commercial considerations (i.e. agreement between AFA, UTAS and Ausindustry) and the needs of the Tasmanian abalone aquaculture industry. A description of the site follows below.

1.1.1 Abalone Farms Australia description

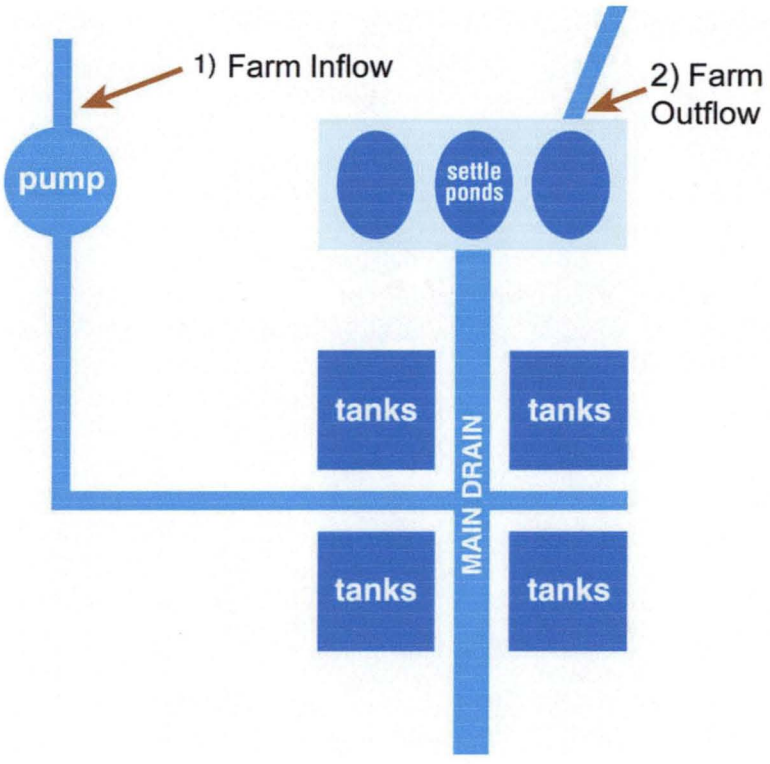

Figure 1.1: Diagram showing the location of AFA in Bicheno, Tasmania, and schematic of the layout of the farm inflow, tanks, sedimentation ponds inflow and sedimentation pond outflow (i.e. same as farm outflow).

Abalone Farms Australia (AFA) is located on the east coast of Tasmania, Australia in the town of Bicheno (Fig. 1.1). A schematic of the farm layout is given in Fig. 1.1. In 2001 AFA commenced the expansion of its operations to enable the target production of approximately 70 tonnes of local greenlip (*Haliotis laevigata*) and blacklip (*Haliotis rubra*) abalone species within a land based culture system. This expansion has been occurring in a series of 4 stages, each of which has seen the addition of 208 abalone grow-out tanks (details below). Currently the farm has completed construction of approximately 410 out of 800 grow-out tanks and the majority of the present study was completed between stages 1 and 2 (i.e. farm size between 208 and 416 tanks).

AFA draws water for use on the farm from the high energy coastline of Bicheno. This occurs through a 6m (H) x 6m (W) x 5m (L) man made pit which has been created in the granite dominated coastline. The bottom of the pit is approximately 6m below sea level and water is pumped without filtration or treatment by two submersible pumps into the 400 abalone concrete grow-out tanks. The tank water flow rate is approximately 25L/minute/tank and is used in a single-use flow-through system. The abalone grow-out tanks are exposed to ambient irradiance intensities and regimes (no shade cloth over farm) and approximately 6.5m x 2.5m in size with varying depths between approximately 0.6m and 1.3m. The tanks hold approximately 10,000L of seawater and are aerated 24 hours a day. Within the tanks are concrete abalone hides and the abalone are fed formulated feed once daily. Twice weekly the each tank is flushed and cleaned of waste. The tank waste and effluent water flows from the tank, into a main drain and then into a sedimentation pond system where solids sediment to the bottom. The sedimentation pond system consists of 3 PVC lined sedimentation ponds linked in series and totalling approximately 5 megalitres of water with residence time of approximately 12 hours when all ponds are utilised. Outflow water overflows from the third sedimentation pond into two 600mm stand-pipes and exits the farm. Over the course of sampling the total farm water flow rate was consistently in the range of 8.6 megalitres per day.

Hypothesis to be tested

1. That the abalone formulated feed leach nitrogen, phosphorus and particulate matter once immersed within the abalone culture tank environment

2. That there is a series of processes which degrade the particulates within Abalone Farms Australia (AFA) as the feed is fed, excreted and transferred to the sedimentation pond
3. There is a diurnal rhythm to the export of nutrients and particulates from an abalone farm
4. That different compartments within the AFA can have a different affect on the production and consumption of nutrients
5. There is a detectable impact of AFA on the marine environment surrounding the outfall
6. The use of seaweed rafts in grow out tanks can reduce the effluent nutrients
7. That while abalone farming does contribute nutrients to the marine environment but should not be considered a major polluter

1.1.2 Thesis structure

This thesis is can be broken into five distinct research sections as listed below:

1. Preliminary studies for the development of an effluent monitoring program
2. Characterisation of abalone farm effluent
3. Environmental Impact Study of effluent receiving environment
4. Techniques for the reduction of effluent nutrients
5. Tasmanian abalone farms environmental monitoring program

Each of these sections has the aims of:

1. To establish the main sources of nutrient production within the farming system and a basis for implementing a long term effluent monitoring program[a]
2. To determine the temporal and spatial variability as well as the key factors affecting abalone farm effluent as it flows through various farm compartments
3. To determine the biological impact of AFA's effluent waters on the intertidal and subtidal areas of the receiving environment
4. To test the capacity of AFA's seaweed rafts to reduce nutrients in the abalone culture tank's effluent [b]
5. To determine whether relationships pertaining to nutrient export that are exhibited at AFA are also exhibited by other farms and the source of any inter-farm variations which may be present

[a] detailed aims of each experiment are provided within chapter

[b] AFA claims Intellectual Property on the development of seaweed rafts (Chapter 4) and therefore detail as to the development of the raft are not given within this thesis.

The structure of this thesis was aligned in accordance with the requirements of the contract outlined between the University of Tasmania, AusIndustry, and Abalone Farms Australia, where broad areas of study were identified for research by AFA and AusIndustry (requirements outlined in AusIndustry grant attained by AFA).

This study gives an overall perspective of the environmental impacts of land based abalone farming in Tasmania, Australia. Comparisons are made between existing marine resource users and abalone farms, allowing resource managers to make informed decisions related to abalone farm environmental management. Additionally tools for the on-going management of the industry are developed along with techniques to reduce any potential environmental impacts.

CHAPTER 2: Preliminary studies for the development of an effluent monitoring program

2.0 Chapter Introduction

Three preliminary experiments were conducted to determine how nutrients were being produced within the abalone farm and how they were varying on a diurnal scale. Production of nutrients is clearly related to the formulated feed, however two possible means of nutrient production were deemed likely. Firstly from the formulated feed leaching or secondly from the degradation of the waste produced on farm. Finally an experiment was conducted to determine the pattern in nutrients exiting the abalone farm's effluent pipe.

The nutrient stability of a commercially available formulated feed is important as on most Australian abalone farms there is usually a delay between the time the formulated feed enters the abalone culture tanks, and the time of consumption by the abalone. This leads us to believe that there may be nutrients from the formulated feed. A number of studies have determined the nutrient stability of abalone diets (Coote et al., 1996; Sales et al., 2003), however all of these experiments have been conducted in experimental systems as opposed to actual abalone culture tanks. Given the recent advances in the abalone feed manufacturing (Fleming et al., 1996) the alternative farming style of AFA (i.e. deepwater culture tanks with strong aeration, relative to the South Australian model of PVC pipes and shallow high flow tanks) and that nutrient stability (of abalone formulated feed) trials have not been conducted within commercial culture systems, it is necessary to test the performance of the formulated feed within the AFA culture tanks. Such an experiment examines the wastage of formulated feed and forms the basis for management of feeding abalone which is clearly the major source of nutrients entering the water.

An examination of the tank waste was conducted to examine if the waste within the abalone culture tanks was a major source of dissolved nutrients that are produced as the waste degrades. Studies in aquaculture facilities such as prawn farming has shown the waste sediment to be a major source of remineralised dissolved nutrients (Boyd, 1992; Boyd and Musig, 1992; Burford et al., 2003; Burford and Longmore, 2001; Burford and Lorenzen, 2004; Burford and Williams, 2001; Hargreaves, 1998). The rates of dissolved nutrient remineralisation are likely to be affected by many processes some of which may include pond dynamics, waste composition, tank cleaning frequency and effluent water temperature. Subsequently proximate analysis and recording of total volumes of the waste in the culture tanks and sedimentation ponds yields information about the relative amounts of nutrient pools and by difference there may be calculation of remineralisation of nutrients.

The temporal variation in nutrient export for a single farm was characterised on a diurnal basis. Monitoring programs around Australia have recorded highly temporally variable results (Council, 2000a; Council, 2000b; Council, 2002a; Council, 2002b) in concentrations of effluent nutrients. Some of this temporal variation is likely to be accounted for by the diurnal variation and time of day for sampling. Active processes of photosynthesis (dissolved nutrient consumption) and remineralisation (dissolved nutrient production) are likely to affect the dissolved nutrient concentrations (Boyd, 1992; Burford, 1997) and as both of these processes may are affected by light regimes. Thus it is likely that dissolved nutrient concentrations vary on a diurnal basis causing temporal variation in results. The broader implications of this research relate to environmental monitoring of nutrients and environmental management. For policy makers to be

effective in providing management tools, there needs to be some depth of research as to the driving forces behind the temporal variation in dissolved nutrients. This will in turn allow sampling and management protocols to be developed and an industry wide comparisons of results. Further comparison of results across farms provides perspective into the most appropriate design of farms with respect to environmental performance.

2.1 Formulated feed nitrogen and phosphate stability experiment

2.1.1 Aim: To determine the rate of particulate matter loss and leaching of dissolved nitrogen (N) and phosphorus (P) from Adam and Amos™ 1mm diet after immersion in Abalone Farms Australia (AFA) tank conditions

2.1.2 Introduction

The main import of nitrogen (N), phosphorus (P) and particulates into an abalone farm is likely to be the feed source. Within Australia the majority of land based abalone farming uses a formulated feed as the food source for their animals. This is in contrast to abalone culture in some countries such as China where they use natural seaweed diets (Nie and Wang, 2004; Zhang et al., 2004). While formulated feeds offer faster growth rates than unenriched natural diets (Bautista-Teruel and Millamena, 1999; Boarder and Shpigel, 2001; Jackson et al., 2001; Viana et al., 1993) and have greater nitrogen and phosphorus contents (Viana et al., 1993) they also tend to be more susceptible to leaching when in water (Jackson et al., 2001). The nutrient and dry matter stability of these feeds once they are in the water is of importance from both an environmental perspective and also from a farm management perspective. This is because lost nutrients from the feed means increased environmental pollution as nutrients which do not reach the abalone are nutrients which ultimately will be delivered to the marine environment. Dissolved

nutrients such as nitrogen and phosphorus are likely to be transported to the marine environment through the outflow of the farm, whilst particulates are likely to be retained within the farm as a solids separation device exists at AFA. Despite retention of the particulate nutrients, there may be remineralised dissolved nutrients if they are broken down by other organisms within the abalone farming system. The loss of nutrients not only will cause environmental pollution, however they may also cause lower feed conversion ratios (feed consumed/weight gained) and increased operational costs per kilogram of abalone produced (Fleming et al., 1996). Improved temporal resolution of the rate of leaching of N, P and particulates from the formulated feed will help to understand how much of the formulated feed is wasted and how much is left available for the animal once consumption begins. Such a study is particularly important for slow, nocturnal, messy feeders such as abalone (Uki, 1981). This is in direct contrast to many finfish aquaculture species which consume formulated feeds almost immediately after it enters the water. Therefore a much more stable formulated feed with respect to nitrogen, phosphorus and particulates is required for abalone farming.

The amount of leaching from a formulated feed is determined by a number of factors associated with both the manufacturing and the environment that these feeds are placed into. In the manufacturing process it is commonly known that the type of binder, particle size and means of production will play a large role in the stability of the aquaculture diets (Fleming et al., 1996), however information as to the manufacturing process and ingredients in Australian diets is closely guarded and generally not obtainable due to commercial sensitivity (Fleming et al., 1996).

With respect to the receiving environment of the feed, the water temperature, water movement and duration of immersion are key factors which will have an impact upon pellet integrity (Myers and Zein-Eldin, 1975; Stewart and Grant, 2002). Generally speaking the longer immersion time, the greater the water flow or greater the water temperature, the less physically stable the feed is likely to be. Issues of feed stability are particularly important for abalone farming given the farmers requirement for maximum growth coupled with the slow nocturnal feeding habits of abalone (Uki, 1981). As the formulated feed is placed in the water during normal working hours, (usually in the late afternoon) it can be expected that there is a substantial degree of leaching relative to other forms of aquaculture where the time taken from immersion to ingestion may only be a matter of seconds (i.e. salmon farming). In the majority of Tasmanian abalone farms for most times of the year, the amount of time the formulated feed remains in the water prior to the onset of consumption is at least two hours during winter and can be up to six hours in summer. Additionally, it has been reported that while 50 - 80% of animals appear during the night (Uki, 1981), as few as 7% of juvenile *Haliotis midae* abalone within tanks actively feed each night (Knauer et al., 1995a). Additionally many abalone farmers also report difficulties in predicting the right amount of feed to add to the tanks (Tasmanian Abalone Growers Association, Personal Communication, 2004) and hence may be overfeeding or underfeeding. Providing sufficient food to maximise abalone growth increases the chance of feed wastage. The above factors combine to cause a level of inefficiency in feeding, partially due to the biology of the abalone, and partially due to the associated husbandry of a nocturnal species.

Measurement of the formulated feed leaching is commonly conducted through immersion of known quantities of the formulated feed (within a container) into seawater. Then at appropriate times removing feed for analysis of the components to be measured. Such a process allows calculation of dry matter loss as well as other components which have environmental significance such as nitrogen and phosphorus. This study examined the stability of formulated feed components once immersed in water. The formulated feed leaching may be contributing to the overall dissolved nutrients within the farming system. Quantification of the exact amounts of leaching may identify if there is further research required into formulated feed nutrient stability as well as giving guidance into the development of management strategies to minimise leaching.

2.1.3 Materials and Methods

Polystyrene 200ml sample jars were modified and used as vessels for testing the physical and chemical stability of the abalone's formulated feed. 65 millimetre diameter circles were cut from the jar lid and 250μm mesh screen was screwed down into the lid (Fig. 2.2).

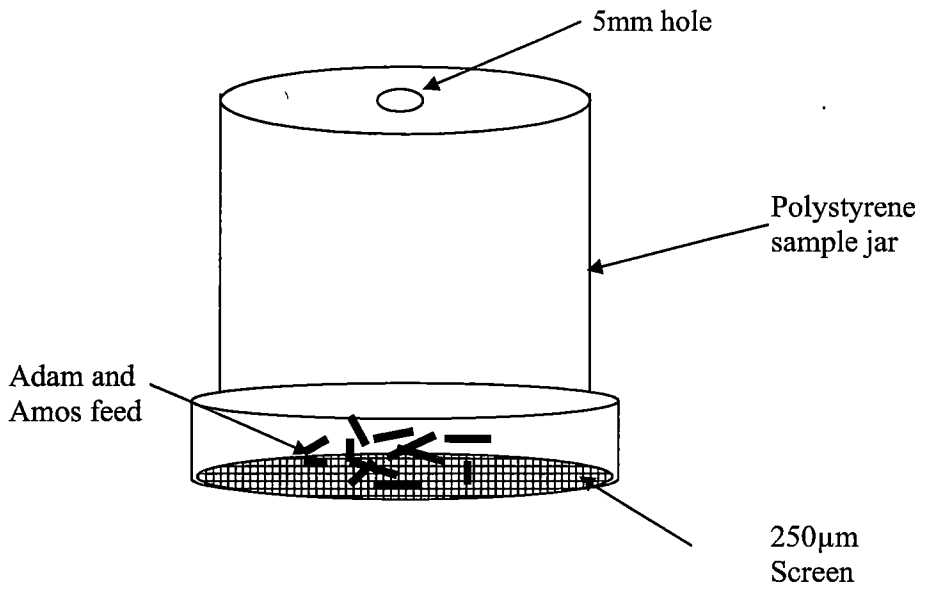

Figure 2.2: Diagram of the modified polystyrene 200ml sample jars used to test the physical and chemical stability of Adam and Amos 1mm formulated feed

A five millimetre wide hole was then drilled into the bottom of the jar to aid water exchange. Approximately five grams of Adam and Amos 1mm formulated feed was accurately weighed into each of 36 jars. The jars were then inverted (mesh faced down) and slowly immersed in seawater in Abalone Farms Australia (AFA) concrete tanks (6.5m x 2.5m) at a depth of 50cm. The labelled jars were placed randomly onto a larger screen approx 85cm in diameter with a mesh size of 2 millimetres. This larger screen was raised on blocks to allow water flow from underneath. A further screen was placed on top of them to ensure no movement of jars occurred within the tanks. The tank used for the experiment replicated the tanks used for growing abalone with ambient seawater, water flow rates, hide numbers and aeration rates were set as per the AFA standard grow-out conditions.

The experiment was conducted during spring (31-10-02) and began at 4pm around the time of the usual feeding as conducted by the farm technicians. No animals were in the concrete tanks at time of experiment. At pre determined times (0, 15mins, 30mins and 1, 2, 4, 7, 10, 14, 19, 24, 26 hours) 3 randomly selected sample jars containing feed were removed and taken to the laboratory for processing. Jars were drained for excess water on 'Teriwipes™' towelling for 15 minutes and then weighed on a tared piece of aluminium foil (shiny side up). The feed samples were then dried in an oven at 105°C for 48 hours and reweighed until constant weight was achieved. The moisture content of the feed was also determined by the same drying process. Nitrogen and metal determination was then conducted. Nitrogen analysis was conducted using a Leco CHNS-932 elemental analyser with cystine as a standard. P along with various other metals were analysed using ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrophotometry). Organic content of the samples was also determined by placing a known weight of sample into aluminium foil and combusted in a Lindberg Blue 828M furnace at 550°C according to the methods outlined in (Franson, 1989)) (Method #2540E). The resulting sample was reweighed and organic content determined by weight difference. Within the tanks temperature was monitored every sampling time where sample jars were removed. This was conducted over the duration of the experiment using a YSI sonde 6600 data logger.

2.14 Results

Figure 2.3 shows a rectangular hyperbola curve fitted to the dry matter loss data over time. There was approximately a 4% loss in dry matter within the first 1hour of the

formulated feed entering the water. This continues to increase until the 4th hour where the rate of dry matter loss begins to decrease with total loss plateauing at around 7 hours at 10.15%. At 26 hours there is an increase in the percentage of dry matter loss to above 12%.

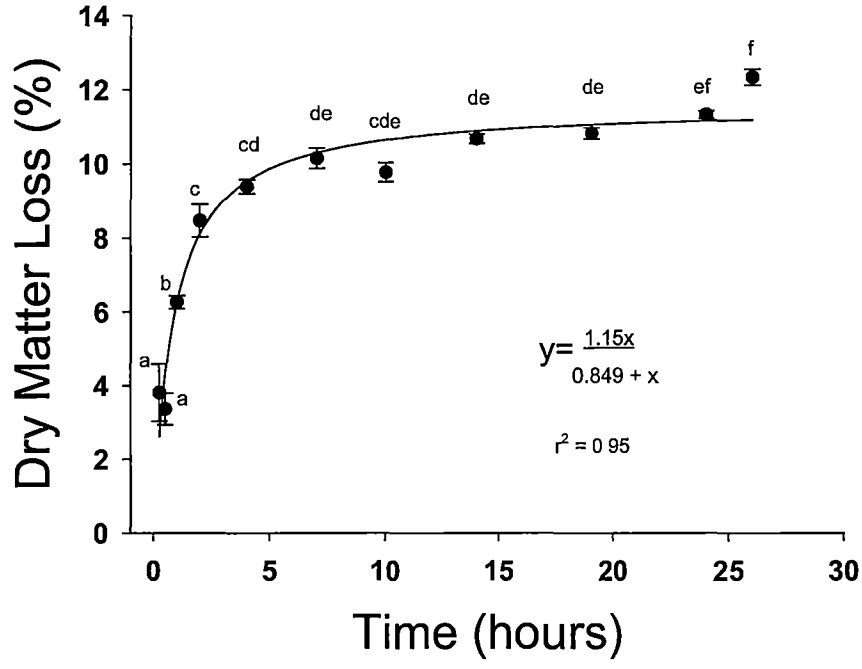

Figure 2.3: Dry matter loss over time for Adam and Amos 1mm commercial abalone diet with immersion in seawater for varying times (mean ± SE, $n = 3$). Values that share a common superscript are not significantly different from each other.

The nitrogen content of the feed decreased over time (Pearsons correlation = -0.592, $P<0.001$, $n=32$) and was significantly different from the initial concentration of 6.3% to 5.5% after 26 hours in the seawater (Fig. 2.4). The trends show that nitrogen loss from the diet generally followed an exponential decay although remained relatively stable

during the first 30 minutes in the water and then the nitrogen content began to decrease exponentially. Between 30 minutes and 7 hours the mean nitrogen content dropped from 6.28 to 5.65% respectively. Beyond 7 hours the rate of leaching began to slow with 0.1% difference in mean percent nitrogen content between 7 hours and 26 hours.

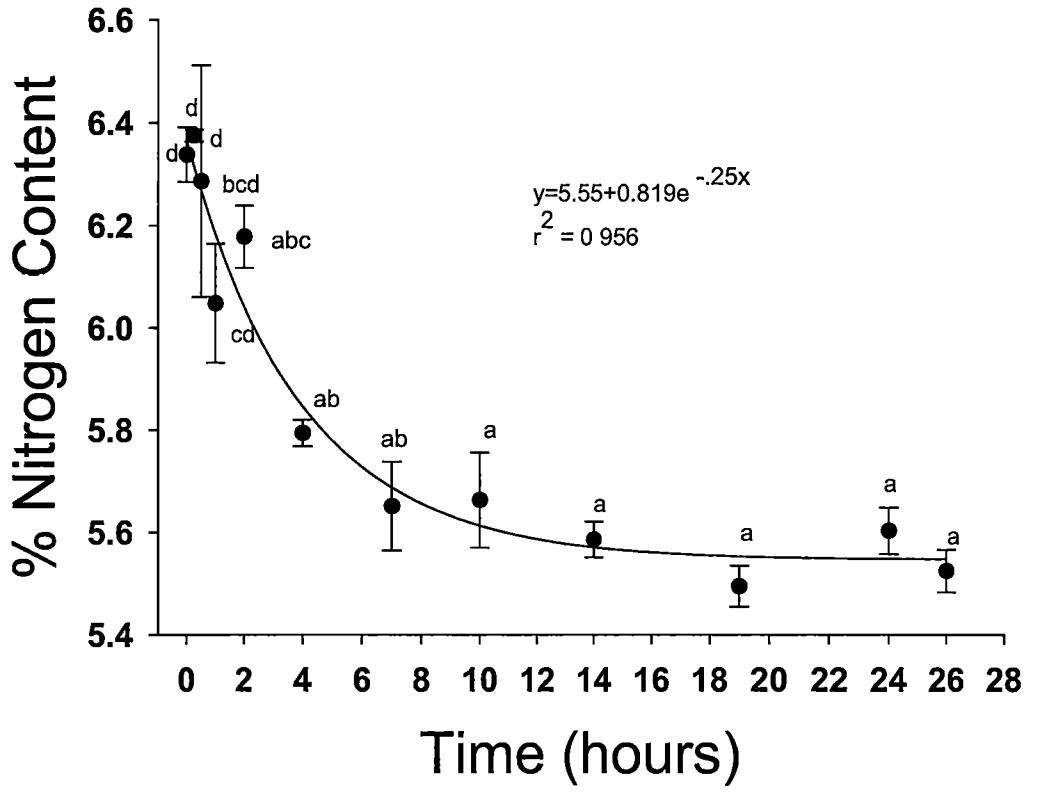

Figure 2.4: Nitrogen content over time of Adam and Amos 1mm commercial diet after immersion in seawater for various times (mean ± SE). Values that share a common superscript are not significantly different from each other.

Phosphorus (Fig. 2.5) showed significant decreases in concentration with time according to an exponential decay curve. The majority of the losses of P to the tank

environment occurred within the first 15 minutes of immersion indicating that post 15 minutes there was good stability of the residual phosphorus.

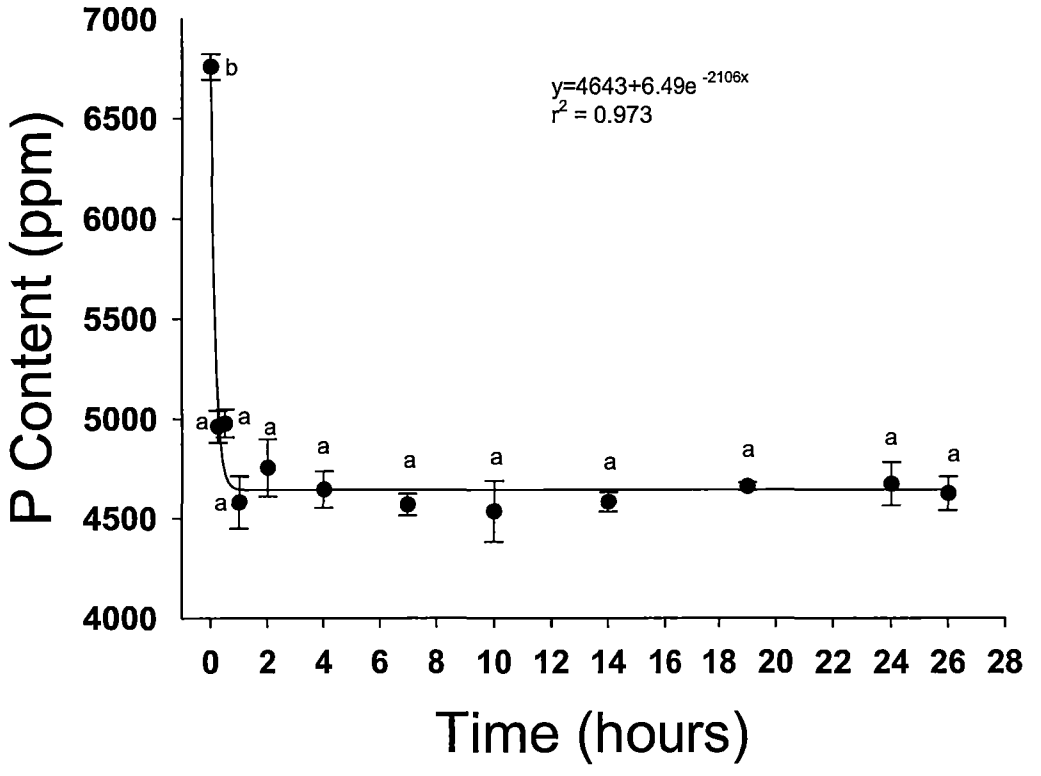

Figure 2.5: Phosphorus content over time of Adam and Amos 1mm commercial diet after immersion in seawater for varying times (mean ± SE). Values that share a common superscript are not significantly different from each other

The temperature fluctuations (Fig. 2.6) within the tank system were in the order of 5.93°C, varying from a low of 11.91°C at 06:20, to a maximum of 17.13°C at 15:20. At approximately 20:20 when logging began, the temperature was 15.49°C compared with 16.33°C the following day at 20:10 indicating a between day variation of under a degree.

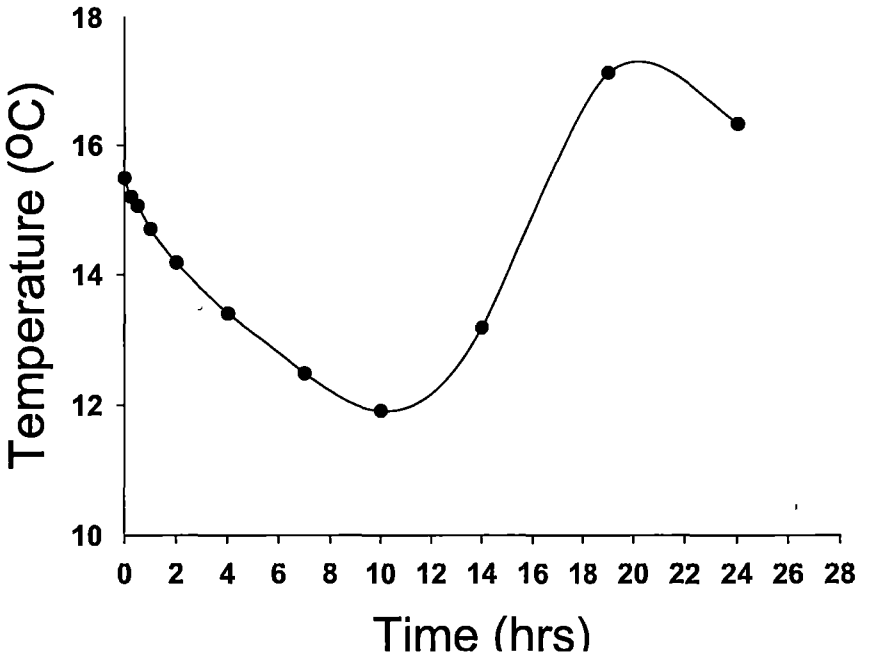

Figure 2.6: Temperature profile within a single abalone grow-out tank over a 26 hour period beginning at 20:20 on the night of the experiment. Temperature was taken at pre determined times during the sampling period. Data taken using a YSI 6600 sonde data logger.

The organic content of the formulated feed showed no discernable trend over the sampled period. The data were highly variable through time and not significantly different over 24 hours ($P > 0.05$).

2.1.5 Discussion

The results of the formulated feed stability trial indicate that some components of the feed are likely to be lost into the tank environment before the animals begin to consume and ingest the feed. Within 4 hours of the feed entering the water, the loss in dry matter (by weight) accounts for approximately 9.4% of the total pellet mass. This finding is similar to another study in which tested two diets which lost between 3.4 and 9.25% dry matter after 6 hours of immersion (Sales et al., 2003). The spectrum of nutrients lost from the diet (as indicated by dry matter loss) is likely to extend well beyond the parameters measured within the present study. It is likely to be a mixture of water soluble components of the feed and/or finer particulates. There is potentially two means by which nutrients can leach from the feed, through dry matter loss and also through losses of water soluble nutrients. It is likely vitamins, supplemental phosphate sources and some metals will be water soluble while proteins, fat soluble vitamins and lipids are more water stable. If the diet is balanced to meet the nutritional requirements of the abalone any differential loss of a particular component may lead to inefficiencies in utilisation and hence further losses of nutrients to the environment.

2.1.5.1 Nitrogen

Marine environments are generally considered to be nitrogen limited (Day et al., 1989) and hence the leaching and release of nitrogen from formulated feed represents some environmental concern. This study showed a loss of 7-8% of the original nitrogen content of the feed within the first 4 hours. On a total farm level, the daily feed rate (for AFA at 15 tonnes abalone biomass) may be anywhere between 30 and 58kg/day (time of

year dependant). Hence assuming consumption occurs predominantly after 4 hours post feeding, the net loss of nitrogen per day from the formulated feed (assume similar conditions to the present study) is likely to be in the order of 150-290g/day (54 - 105kg/year) of nitrogen (before feeding commences). When we considered the daily feed rates and the actual load of nitrogen within the formulated feed, the actual amount of nitrogen lost through leaching may contribute a significant portion of the total farm N budget. However depending upon the discharge environment and the water usage on the farm this amount of nitrogen may or not represent a threat to the marine environment. In the current situation the 100kg of nitrogen is unlikely to represent any significant environmental threat to the Bicheno coastline when we consider the loads of nitrogen from sewage farms which may be orders of magnitude higher.

2.1.5.2 Phosphorus

Phosphorus concentrations of the diet decreased markedly within the first 15 minutes of the diet entering the water. Approximately 31% of the phosphorus in the diet was lost during the first 15 minutes after which the diet remained relatively stable throughout the remainder of the sampling period. Trials conducted by Sales *et al.* (2003) showed similar rates of phosphorus leaching to the present study in the first hour, yet Sales *et al.* (2003) found greater rates of leaching of up to 60% of P were exhibited in some diets over the 24 hour period. The greater rate of leaching found by Sales *et al.* (2003) may be a function of temperature which was kept at 18°C in the Sales *et al.* (2003) experiment. In the current study, the water temperature fluctuated approximately 5 degrees over the 26 hour period. It is possible that the water temperature had an effect on

the stability of the formulated feed. The results of this trial represent the stability of the 1mm Adam and Amos diet on a day where water temperatures ranged between 12 and 17°C. Over the course of the year the tank water temperature ranged between 11-21°C, and hence the results of this trial are perhaps indicative of the diet stability during conditions which were more favourable for pellet integrity (i.e. not a worst case scenario). Overall it seems that up to 30% of the P in abalone formulated feeds is likely to be wasted and not reach the abalone at all. Instead it enriches the effluent waters of AFA causing an unnecessary environmental pollution.

2.1.5.3 Organics

The organic content of the diet appeared to show no significant trends throughout the sampling period. There was a large degree of variability between the replicates. It is possible that there was a decrease in the organic content of the formulated feed given that all mean values were below the initial organic concentration of 60%. Certainly it is reasonable to assume that there was some loss of proteins, lipids, carbohydrates and other organics; however it is unclear as to the proportions of organics lost relative to the loss of the inorganic component. Some of the variability may lie in the sensitivity of the weight difference method used to determine organic content.

It is possible that some of the losses through leaching may be overcome by automatic feeders which feed later in the evening rather than during normal working hours. Despite this many farms are reluctant to move towards an automated system due to the possible feed wastage and associated costs, along with a lack of 'hands on' animal

husbandry associated with them. In addition it has been noted by (Knauer et al., 1995b) that abalone appear over a 6 hour period after dark which would suggest that the use of automatic feeders would not solve the problem of phosphorus loss, however the nitrogen and dry matter losses may be reduced using this method (i.e. majority of leaching occurs within the first 6 hours of the formulated feed entering the water).

2.1.6 Conclusions

Overall the formulated feed appeared to be relatively stable over the period of sampling. The loss of dry matter from the pellets seems to be preventable if feeding was to occur closer to darkness as the majority of the dry matter was lost during the first 6 hours of immersion. Associated with this dry matter loss would be trace metals, vitamins and proteins and lipids which provide the essential nutrients for fast and healthy abalone growth.

Losses of nitrogen equated to 15% of the total nitrogen content of the pellet over 24 hours which is likely to be an acceptable loss in from a farm husbandry and nutritionist perspective. In terms of the environmental implications we have noted that whilst at current feeding rates 100kg per annum is not a large amount of nitrogen to be delivered to the Bicheno coastline, if production was to increase ten fold (i.e. to the projected production capacity of the farm at full development), then there may be some source for environmental concern. Again much of this waste it seems is preventable by feeding later in the evening (i.e. closer to darkness) offering possible benefits to the abalone in terms of higher nitrogen content in their food.

Of concern to the feed manufacturers is the large loss of phosphorus which occurs within 15 minutes of the feed entering the water. While this appears to be a common problem based upon the results of other studies (Coote et al., 1996; Sales et al., 2003), it would seem to be causing daily pulses of dissolved phosphate. While this may not be a huge environmental problem, it certainly seems to be something which is avoidable. Feed companies should consider assessing whether the phosphate lost is supplemental phosphate or other phosphate bound within the base diet. As the phosphate is clearly not reaching the abalone it seems pointless to be supplementing and causing unnecessary environmental pollution. For the current position of the industry, the feed appears to be suitable in terms of all other measured components. Perhaps technology examining the actual delivery mechanism to the abalone culture tanks may also be a means to further develop abalone feeding efficiency.

2.2 Particulate waste experiment

2.2.1 **Aim**: To determine the nitrogen (N) and phosphorus (P) composition and total nutrient pools of tank and sedimentation pond sediment that is generated between periods of tank cleaning at Abalone Farms Australia.

2.2.2 Introduction

It is important from an environmental perspective to understand how efficient the abalone are in utilising the artificial feed as this will also give an indication of how much waste is generated in the process of consumption; this waste being a potential source of nutrient export to the marine environment. The waste generated by the abalone may be in either a particulate or dissolved form. Within an abalone farm (that possesses a sedimentation pond) the pool of particulate waste represents a source of organic matter and hence potential nutrients that may eventually be discharged into the marine environment. Commonly the particulates waste within an abalone farm periodically reside within the grow-out tanks between cleaning events, however after cleaning these particulates are washed down into the drains and ultimately make their way down to the sedimentation pond. A proportion of these sedimentation pond particulates will be decomposed and remineralised into the water column as nutrients. Some of these remineralised nutrients may be taken up by organisms within the farm system while some may be exported from the farm as dissolved nutrients in the effluent. An understanding of the biochemical composition of the particulate waste generated within the grow-out tanks

will not only allow an understanding of how efficiently the food source is being utilised, but also the 'potential nutrients' held within the waste may also be characterised. There are a number of means to assess the nutrients within abalone waste; each with their own costs and benefits.

A possible method to determine the composition of particulate waste (generated by an abalone feeding on a given diet) is a digestibility trial which collects the solid waste products of the abalone for analysis. While a typical nutrition digestibility trial actually aims to determine the digestibility of a given diet, it will also characterise the particulate waste by determining faecal composition. Unfortunately abalone faeces are very difficult to characterise as they are both delicate in nature and rapidly leach into the water column (Anderson, 1988; Fleming et al., 1996; Wee et al., 1992). In addition to the above, contentious issues exist surrounding the choice of digestibility markers (Fleming et al., 1996). While such a trial should give a reasonably accurate description of the faecal composition from a particular diet under the given conditions (if performed with the appropriate measures i.e. ice collectors, low flow rates), the trial fails to give a real life description of the particulate waste generated under commercial culture conditions (i.e. including particulate waste generated from the consumption of naturally available diets (e.g. diatoms) and also uneaten formulated feed). Accounting for these factors will provide a much more accurate description of the particulate and subsequent potential nutrient pool.

Another possible technique to assess particulate waste products is sampling the particulate waste directly from the grow-out tank floor (e.g. on site at AFA) and analysing this material for its constituents. Such an experiment would not only be

collecting abalone faeces (artificial and natural diet), but also uneaten feed, particulates from the farm inflow waters, and potentially any other micro organisms which have colonised or broken down the organic material within the tank. The collection of this waste is likely to give a much better description of the actual waste and hence its potential capacity to release nutrients. The main disadvantage of sampling the tank particulate waste directly is that there is likely to be leaching from the waste material into the water column causing some unquantified variability (error) in the results. Unlike the digestibility trial, this leaching cannot really be controlled (e.g. through cooling of faeces to minimise leaching) or accounted for; however, to minimise the amount of leaching, collection of particulate waste as soon as possible after excretion by the abalone is desirable. Another problem lies in the collection of the particulate waste as aquaculture waste is generally quite dilute (Cripps and Bergheim, 2000) and hence collection of just the waste is often problematic as large volumes of water need to be filtered. Considering the issues discussed, a means of directly sampling the particulate waste within the tanks was developed to determine how much particulate waste is generated by the abalone when consuming formulated feed in a commercial culture environment (described in 2.2.3. Materials and Methods below).

As tank particulate waste is flushed from the tanks during cleaning, the solid waste is collected within AFA sedimentation ponds and hence is a source of nutrients held within the farm. In addition to analysing the tank particulate waste material, analysis of the sedimentation pond sediment may also yield important information as to the concentration of nutrients held within the sedimentation ponds and hence the capacity of the sedimentation ponds to 'produce' nutrients.

2.2.3 Materials and Methods

Three tanks representative of standard AFA grow-out conditions were chosen to determine the composition of the waste generated during culture conditions over a three day period (AFA standard number of days between cleaning). Tanks were chosen according to animal numbers (4000) and animal size (40-60mm animals). An additional 3 newly finished tanks were chosen as controls which contained no animals but the same water flow regime. These tanks were employed to determine if the incoming water contained particulates and hence contributed to the tank particulate waste collected. Standard AFA practices have the animals being fed daily and tanks cleaned twice weekly. The day before the experiment commenced (day 0), the 6 chosen tanks were cleaned and flushed of all particulate matter. No feed was placed into the three experimental tanks on the night prior to the experiment. A known amount of feed was then weighed into a 4L container and fed to the animals by AFA farm technicians over three consecutive days (days 1 to 3). The rations for each tank were determined by the AFA farm technicians and were standard farm practice based upon previous feeding history, stocking density and water temperature. The feeding during this experiment was designed to simulate normal every day practices on the farm and hence generate a 'typical' quantity and quality of particulate waste. At the end of the three days, the amount of unused feed was weighed and the amount fed into the tanks calculated by subtraction. On a daily basis (approximately 7-9am) a section of each tank floor area equating to 1/5 (Fig. 2.7) of the total floor surface area was sampled for particulate waste by siphoning and the waste appeared to be uniformly spread around the tanks. The area sampled was free from hides and no movement of hides occurred as this would have likely changed the results of the

experiment. This is because the staff at AFA have noted that the abalone take 1-2 days to begin feeding again after a disruption of the tank occurs (i.e. moving hides or anesthetising for grading purposes).

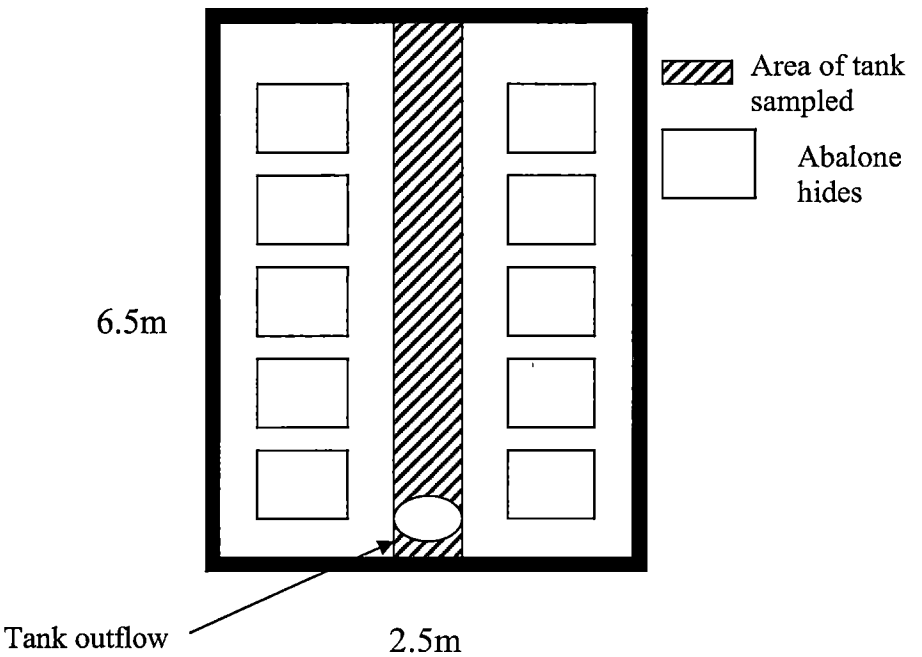

Figure 2.7: Schematic of the area sampled for tank sediment within the standard AFA abalone grow-out tanks. Calculation of total tank sediment contents assumes the area sampled was representative of the whole tank.

To ensure the faeces produced from the allotted feed were collected, feeding did not occur on the third night and an extra day of siphoning was included on the 4th day. Collected waste was concentrated by gravity filtration onto a 250μm screen. Finer screen sizes were trialled; 63μm and 125μm; but were not practical for collection due to clogging and their inability to drain. Once siphoning had been completed and all waste

accumulated, the screen was left to drain of excess water. After initial draining the screens were gently shaken and left for a further 15 minutes.

Samples of sedimentation pond sediment were taken from various points around the rectangular shaped sedimentation pond by the use of 43mm Perspex corers. A total of nine cores were taken from the pond which was made up from three samples along three different transects lines. The transect lines ran perpendicular to the longer axis of the pond, and were at equal distances along this longer axis (Fig 2.8).

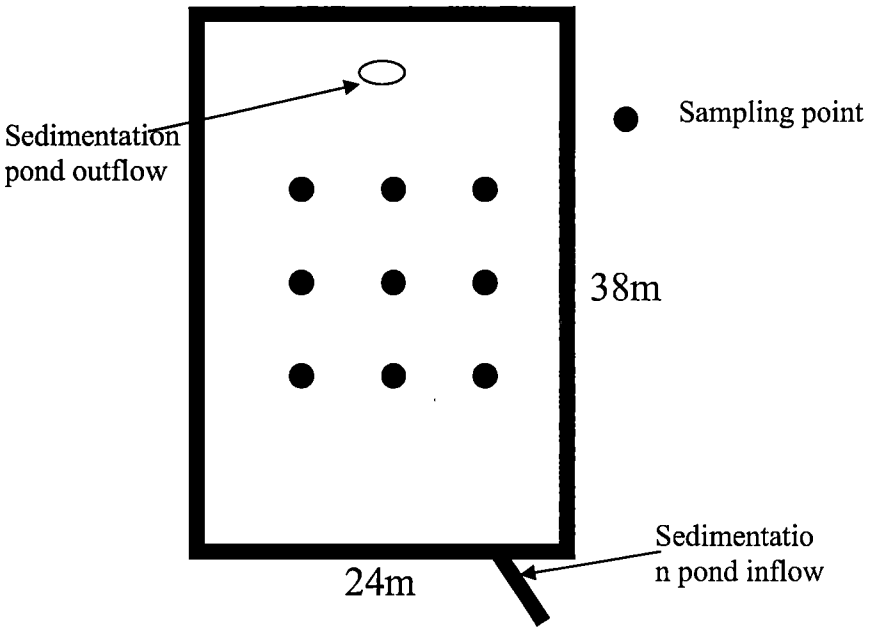

Figure 2.8: Schematic of the sediment sampling points within the sedimentation pond

The samples from both ponds and tanks were stored at –20°C. Upon completion of sampling, random sub-samples were dried at 105°C for a period of 48 hours. After constant weight was achieved, the resulting dry material was ground using a mortar and pestle. Analysis for P (ICP-OES), organic content (2540E (Franson, 1989)) and nitrogen

analysis (Leco CHNS-932 elemental analyser – cystine standard) was performed on the waste samples. Organic content of the feed was estimated by placing a pre weighed and recorded amount of feed sample into aluminium foil and combusted in a Lindberg Blue 828M furnace at 550°C according to the methods outlined in (Franson, 1989)) (Method #2540E). The resulting sample was reweighed and organic content determined by weight difference.

2.2.4 Results

A clear progression in decomposition of the formulated feed can be seen between the original feed composition, the post consumption feed composition and further the sedimentation pond waste composition. Concentrations of nitrogen, phosphorus, organic content and carbon all decrease by approximately 50% between each level of sampling (i.e. feed, tank, pond) (Table 2.1).

Table 2.1: Chemical composition of artificial diet, tank particulate waste and sedimentation pond sediment (mean ± SE). Values that share a common superscript are not significantly different from each other

	Artificial Diet (data from water stability trial)*	**Tank Particulate waste****	**Sedimentation pond particulate waste*****
Nitrogen (%)	6.34 ± 0.08^{c}	3.38 ± 0.24^{b}	1.93 ± 0.18^{a}
Phosphorus (ppm)	6758 ± 64^{c}	4694 ± 110^{b}	2549 ± 64^{b}
Carbon (%)	38.4 ± 1.4^{c}	17.4 ± 1.2^{b}	12.0 ± 0.54^{b}
Organic content (%)	59.8 ± 5.5^{c}	35.4 ± 3.95^{b}	22.5 ± 2.2^{b}

Results above are:

*mean of three samples

**pooled mean of three tanks (each tank had three analytical replicates)

***mean of nine sampling points

The control tanks did not yield any particulates in the > 250μm fraction as relatively calm conditions were observed over the three test days (Ho, Personal observation).

The total pools (i.e. pool refers to particulate waste concentration multiplied by volume of waste sediment) of N, P, organics and particulates decreases roughly by a factor of 6 between the artificial diet and the tank particulate waste pools (Table 2.2). Assuming an annual feed budget of 20,000kg (Chapter 3), approximately 6000kg of particulate waste is likely to be produced (> 250μm fraction only) primarily in the form

of uneaten feed and faeces (uneaten feed is estimated to make up 10- 20% of total amount fed as estimated by AFA technicians (Personal Communication, 2004). This tank particulate waste would contain 2197 kg of organics, 1092 kg of which was bound as carbon, 213kg as nitrogen, and 29kg as phosphorus.

The total pools (particulate, organic, N, P, C) held within the sedimentation ponds was difficult to determine due to the difficulty in measuring the total volume or weight of sediment within the ponds. This is because particulates settled in a spatially uneven nature around the sedimentation pond (Ho, Personal Observation 2004).

Table 2.2: Estimated annual pool sizes of various components of the artificial diet, tank particulate waste and sedimentation pond particulate waste

	Annual input of artificial diet (kg)	**Tank Particulate waste (kg)**	**Sedimentation pond sediments (kg)**
Total Particulates	20,421 kg*	6,278 kg*	
Organic Pool Size	12,211 kg	2,197 kg	
Nitrogen Pool Size	1,225 kg-N	213 kg - N	
Phosphorus Pool size	138 kg – P	30 kg - P	
Total Carbon Pool size	7841 kg – C	1092 kg - C	

*Figures based on February 2004 farm data, where daily farm feed rate = 55.9kg formulated feed, daily tank particulate waste generated = 17.1 kg

2.2.5 Discussion

The organic carbon, N and P concentrations found in feed and then in the particulate wastes within the farming system can be seen to decrease between the tank and settlement pond compartments. By the time the formulated feed exits the abalone as faeces and is further mixed with uneaten feed and other detritus it contained approximately 50% of the organic, N, P and C concentrations of the original feed. Comparisons of concentrations while useful as a snapshot, really need to be placed in context by considering them as total pools (i.e. concentration multiplied by volume).

The results indicate that about 70% of the total feed is not accounted for in the particulate wastes, presumably retained by the abalone or lost to other processes (discussed below). The other 30% is left uneaten or excreted by the abalone as particulates into the tanks. The portion of this particulate waste that accumulates in the sedimentation ponds was not quantified (due to the high level of variation in the depths of sediment and irregular distribution of the sediment deposits within the pond), so the possible change in pool size cannot be accurately calculated (discussed below). The results of this experiment must be interpreted with caution as a number of limitations apply.

While there are definite changes between the composition of the artificial diet and material collected from the tank floor, the differences cannot be attributed solely to the abalone. Results show that between 60-87% of the constituent pools (i.e. all measured components) were lost between the feeding of the abalone and collection of particulate waste (i.e. difference between artificial diet pool and tank waste pool). In spite of the fact that there is likely to have been uneaten formulated feed within the tank particulate waste,

and this is likely to have a higher composition of nutrients than the faeces (hence increasing the concentration of nutrients within the particulate waste (Chapter 2.1)), it is likely that the results of the present study underestimate the N and all other measured components lost from the diet due to feeding by the abalone. The reasons for underestimation may include the sampling regime, where the screen size used to collect the particulate waste was 250μm and hence the fraction < 250μm was not collected. Unfortunately this was unavoidable due to the impracticalities of using a finer mesh size. A lesser source of error for the pool of particulates within the tank system being underestimated may be due to the tank particulate waste being collected during the early hours of the morning. Given that there may have been a number of hours between the diet entering the water, excretion by the abalone and particulate waste collection, some degree of leaching is likely to have occurred with organics, N, P, and C being released into the water column (Chapter 2.1) (Wee et al., 1992). However, our methodology for faecal collection was within 12-16 hours of feeding which is the methodology used in a number of digestibility experiments (Montano-Vargas et al., 2002; Sales and Britz, 2001; Sales and Britz, 2002).

While the loss or gain of nutrients in the particulate waste between the tanks and sedimentation pond is undescribed (due to lack of quantification of volume of particulates in sedimentation pond), there is likely to be decreases in the pools of constituents caused by the utilisation and breakdown of the particulate waste by organisms living within the farming system. Organisms such as bacteria (Moriarty, 1997), filter feeding bivalves (Cheshuk et al., 2003; Lefebvre et al., 2000), fish (Qin et al., 1995), polycheate worms (Olivier et al., 1995) and a host of others may be able to directly utilise aquaculture

particulates for growth hence causing the reduction in the total pool of sediment particulates. Both within the sedimentation ponds and drains, all of the above organisms can proliferate and were periodically observed. Despite this, it is important to note that while these organisms are not quantified and thus not represented in the total particulate nutrient pools within the system. They do represent 'nutrients' within the system and unless harvested or removed will exit the system at some stage but perhaps in a different form. Furthermore any dissolved nutrients released from particulate waste may in fact be recycled by organisms capable of utilising the nutrients (photo and chemoautotrophs) either staying within the system biologically bound, exiting through the farm outflow or perhaps being consumed by the abalone and further excreted as waste.

It is also important to note that while the formulated feed within the farm system represents the largest input of particulates it is not the only source. Lesser inputs of particulates may be derived from farm inflow water or terrestrial particulates carried into the system through rain and wind. The amount of particulates imported into the farm from the farm inflow waters is likely to be affected by the coastal sea conditions, where rough days are likely to cause greater resuspension of particulates at the point of intake relative to calm sea days. This is likely due to coastal wave action combined with the build up of seaweed and particulate matter in the granite intake pit (dimensions = 6m x 6m x 5m that is connected to the ocean through a narrow channel). As the sedimentation ponds are level with the ground, they are susceptible to increased particulates through periodic input of terrestrial matter by wind and rain events.

Nevertheless the total nitrogen content of the sedimentation pond particulate waste is likely to be low (less than 200kg – i.e. tank waste pooled N content) despite the

relatively high particulate volume (approximately 6000kg based on estimated annual tank particulate waste pool size) which was high in inorganics (approximately 60%) presumably due to the consumption of organics by biota and, possibly, the periodic input of terrestrial clay (through construction works run off). The input of clay into the sedimentation ponds was likely to be a relatively small input given the large amount of tank particulate waste flushed into the sedimentation ponds.

Overall this experiment has shown that at least 30% of the formulated feed entering the tanks is likely to become particulate waste which is flushed into the sedimentation ponds twice per week when the tanks are cleaned. The net concentrations of particulates decreases by approximately a 50% factor for all constituents measured between the formulated feed, tank waste and sediment pond waste. The reduction in concentration of constituents between the tank waste and sediment pond waste indicates a loss of nutrients which is likely to be in the form of either leaching or consumption by organisms within the farming system. Within the settlement ponds, organisms capable of active decomposition of the sediment component include, mussels, bloodworms, sea hares, bacteria, abalone, and crabs, all of which were observed over the duration of the project.

2.3 Diurnal nutrient rhythms

2.3.1 **Aim**: To characterise the diurnal variation in total abalone farm nutrients loads exported to the marine environment

2.3.2 Introduction

As abalone aquaculture continues to produce more abalone, it also is improving its efficiency and cost effectiveness by ensuring that as much feed and associated nutrients are retained within the abalone as possible. Coupled with this increase in production also needs to be the environmental research which explores the mechanisms to monitor and regulate the industry. The use of environmental monitoring programs is one such means as if they are conducted appropriately, monitoring programs can accurately assess the environmental performance of an abalone farm. Currently there are a number of small monitoring program around the states of Victoria and South Australia. While these monitoring programs differ greatly in terms of the amount of information they collect, they both aim to ensure that trends in the activities of an industry are not affecting the marine environment. The monitoring programs in Victoria and South Australia both monitor effluent nutrient concentrations however there is still vital information required to place the results of these programs into context. One of the potential flaws in both the Victorian and South Australian monitoring programs is that they both rely on the assumption that nutrient concentrations are consistent over a 24

hour period and that a sample taken during any time of the day is representative of the abalone farm on that given day. In fact it seems there may be a number of steps which can cause variation in the nutrient production rates and amounts over a diurnal cycle. Studies of numerous aquaculture grow out ponds have shown a strong relationship between photosynthetic activity and ammonium concentration within the both culture and settlement ponds (Krom et al., 1989; Tucker et al., 1984; Tucker and Van Der Ploeg, 1993). Whilst abalone at AFA are not cultured in ponds but rather small concrete tanks the relatively long residence time (7 hours) and low flow of the tanks does allow large mats of algae to grow on the walls of the tanks (Ho, Personal observation 2004).

Subsequently daily variations in nutrients are likely to be affected by irradiance which in turn affects temperature within the tanks. Temperature varies on a daily basis within land based aquaculture grow out ponds (Culberson and Piedrahita, 1996) where warmer temperatures are experienced during the daytime hours simply due to solar irradiation and ambient air temperature. Daily variations in temperature are likely to affect the processes of production and consumption of nutrients differently. Temperature is likely to cause variation in the production of nutrients from sediments. Studies have shown that temperature plays a key role in the flux of nutrients from sediments into the water column (Aller and Benninger, 1981; Klump and Martens, 1989); however the effect of temperature is less pronounced in sediments receiving high organic loadings (Hargrave et al., 1993; Holmer and Kristensen, 1996). Temperature is likely to effect the consumption of nutrients through the rate of photosynthetic activity and therefore nitrogen and phosphate utilisation (Christensen et al., 2003; Hargreaves, 1997; Lefebvre et al., 2001). Similarly, the daylight hours are likely to cause variation in the nutrient loads exported

from farms as photosynthetic activity decreases during night-time hours. Another factor to consider is the abalone physiological activity. Abalone are nocturnal feeders (Uki, 1981) and hence are most active during the night time. Subsequently we would expect elevated dissolved and particulate waste products to be excreted during the night time with particulate waste being excreted for up to 60 hours post feeding (Shipton and Britz, 2001). As a consequence of the complex temporal dynamics of nutrient cycling within an abalone farm the collection of a single sample is likely to give a misleading indication of the total nutrient discharge load.

For environmental monitoring purposes, where single "snapshot" samples are usually taken on farms, sampling at different times during the day may produce different estimates of nutrient discharge rates. If there are temporal cycles in nutrient discharge then any sampling regime designed to monitor environmental performance must take this into account. A simple random sample will not give an adequate assessment of a diurnal cycle. Knowledge of how a farm behaves with respect to daily nutrient export will allow some level of perspective to be added to the results of samples taken at any given time of day. For example how representative is a sample taken at 9am of entire day's nutrient load? Further with respect to monitoring programs, the diurnal pattern of the farm's nutrient export may provide an indication as to the appropriate time to sample. This study investigates the diurnal pattern of nutrients exported from Abalone Farms Australia.

2.3.3 Materials and Methods

The trial was conducted during late summer at Abalone Farms Australia (AFA) (Bicheno, East coast Tasmania, Australia). Standard AFA tanks (dimensions = 6.5m x 2.5m x 1.5m) conditions of aeration and water flow rates (total farm = 8.6 megalitres flow rate) were maintained and remained constant (variable speed drive pump and aeration blower settings were recorded for water flow and aeration respectively) throughout the duration of the 24 hour trial. There were a series of 24 newly finished tanks which had been commissioned prior to the commencement of the experiment. Standard conditions were also maintained within these tanks. Over the duration of the experiment no cleaning of tanks occurred. It was determined through previous work that although cleaning does increase the nitrogen export of farms (Ho et al., 2003), it is likely to be relatively low when we consider total nitrogen exported annually. Our maximum concentration of ammonium was approximately 10μm. During cleaning concentrations can be expected to increase by a factor of 30-100% as shown in Ho *et al.* (2003) and unpublished PhD data. Assume worst case scenario of 100% difference between cleaning and non cleaning.

Our calculations suggest that if cleaning were to occur at a rate of 2 times per week over course of the year this would equate to under 30 kilograms of nitrogen. i.e.:

Annual difference:

= (Conc. Diff. x Tank Volume x Number Of Tanks x Molecular wt of N x Cleaning days)/10^9 (conversion between μg to kg)

= (10μm x 10,000L x 200 tanks x 14 x 104 days)/10^9

=29.1Kg

Feeding was carried out as per standard practice between 3-5pm on the day of sampling. Feeding commonly occurs on other abalone farms once per day and at approximately the same time. Triplicate samples of nutrients were taken from the farm inflow and farm outflow using 10ml polypropylene Sarstedt tubes (cat # 60.9921.819). Samples from the intake were taken using a Niskin water sampler at the farm inflow and samples at the farm outflow were taken from a point past the sedimentation pond outflow but before the water was returned to the ocean via a single 600mm pipe (Figure 1.1). Samples were analysed for nitrite, nitrate, phosphate and silicate on a Technicon® AAII autoanalyser as outlined in (Plaschke, 1999). Silicate was measured as preliminary investigative samples of effluent indicated unusually high concentrations. Further investigation into the diurnal fluxes was sought. Ammonium was analysed as per the methods outlined in (Watson et al., 2004). Water column particulates(measured as Nephelometric Turbidity Units (NTU)) and dissolved oxygen (% saturation) were measured using a YSI Sonde 6600 data logger. On three separate days the logger was placed for 24 hours in each of the farm inflow waters and farm outflow waters logging every 10 minutes (i.e. 3 x 24 hour periods in total for each point).

Statistics for all nutrient sampling were conducted using a two tailed paired t - tests and probabilities less than 0.05 ($P<0.05$) were considered significant. Comparisons between the farm intake and farm outflow were made for each sampling point through time. Pearsons correlations were also conducted for each nutrient comparing the similarity in trends between the farm intake and farm outflow.

2.3.4 Results

For all nutrients there were greater concentrations at the outflow compared with the intake waters ($P<0.001$, t = 14.09, $n=13$). Dissolved nitrogen (NH_3+NO_3+NO_2) concentrations at the intake did not exceed 1µM over the period sampled (Fig. 2.9).

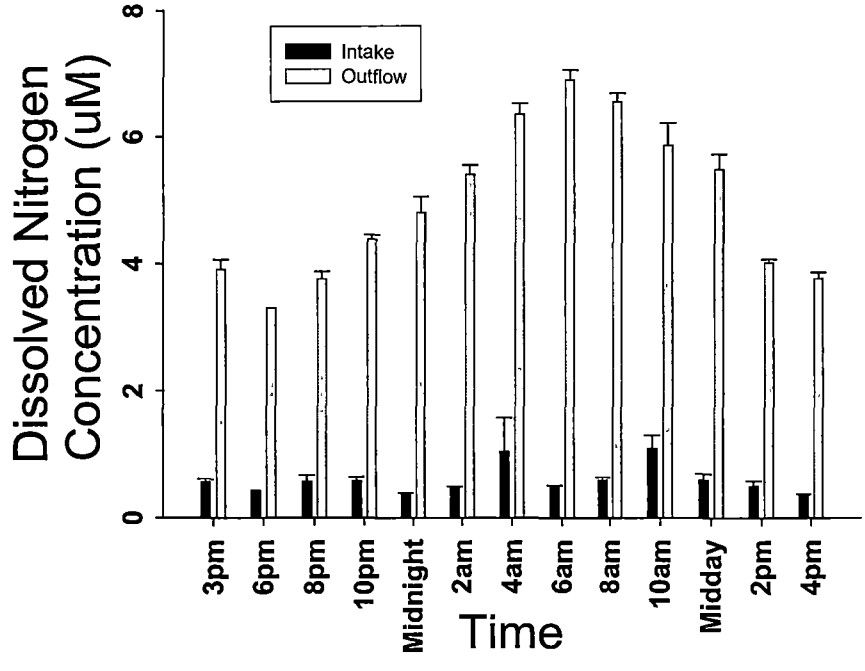

Figure 2.9: Dissolved nitrogen (NH_3+NO_3+NO_2) concentrations over 25 hours for the intake and outflow waters of Abalone Farms Australia. Mean ± SE (n=3)

The outflow waters showed an average increase in dissolved nitrogen concentrations of 834% with a peak in average concentration occurring at 6am or 15 hours post feeding. There was no correlation between the intake dissolved nitrogen concentration and the outflow dissolved nitrogen concentration over the period sampled (Pearsons R = 0.096, P= 0.561, n= 39). The dissolved nitrogen at the farm intake was, on average, comprised of 47% ammonium, 41% nitrate, and 13% nitrite, while the farm outflow it was 87% ammonium, 9% nitrate and 4% nitrite.

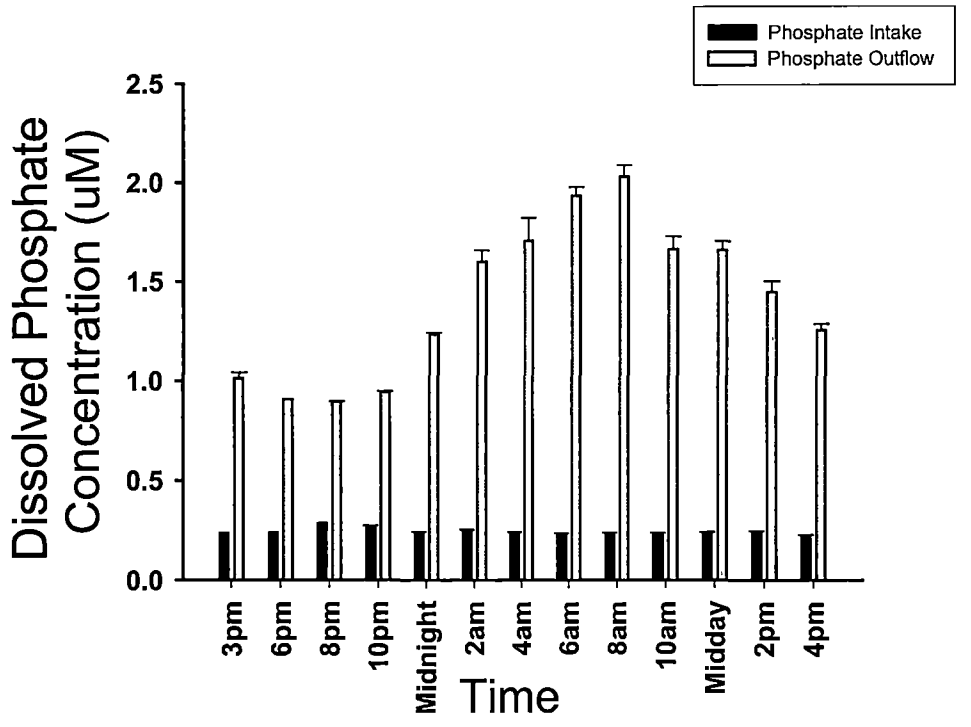

Figure 2.10: Phosphate concentrations over 25 hours for the intake and outflow waters of Abalone farms Australia. Mean ± SE (n=3)

Similar to dissolved nitrogen, the phosphate concentrations at the outflow were on average 580% greater ($P < 0.001$, t = 10.42, $n = 13$) than the concentrations at the inflow (Fig. 2.10). At the intake the concentration of phosphate remained below 0.5 µM while for the outflow the concentrations ranged between 1-2 µM with a peak in mean concentration at 8am. The intake and outflow concentrations were significantly negatively correlated (Pearsons R = -0.541, $P = 0.001$, n=36).

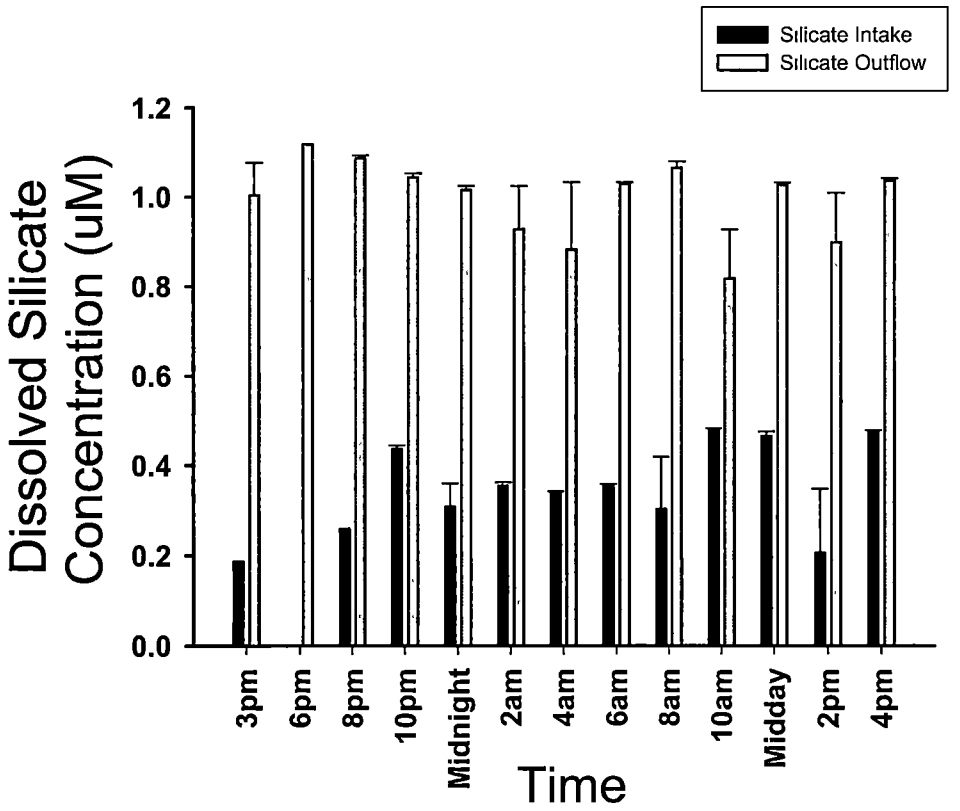

Figure 2.11: Silicate concentrations over 25 hours for the intake and outflow waters of Abalone farms Australia. Mean ± SE (n=3)

Silicate concentrations at the farm outflow were approximately 1μM which was a 100% increase on the average concentrations ($P < 0.001$, t = 11.22, $n = 13$) at the inflow (0.4-0.5μM) (Fig. 2.11). The 24 hour temporal trends in Si concentration observed at the intake were not related to those the outflow (Pearsons R = -0.300, $P = 0.075$, $n = 36$).

Turbidity measured as nephelometric turbidity units (NTU) showed little evidence of a diurnal trend at the both the inflow and outflow. Figure 2.12 shows the spread of results attained over three 24 hour periods at both the intake and outflow. At the inflow the average turbidity over the three days sampled was 0.45 ± 0.04 ($n = 432$). The outflow showed relatively lower average turbidity of 0.27±0.02 ($n = 441$).

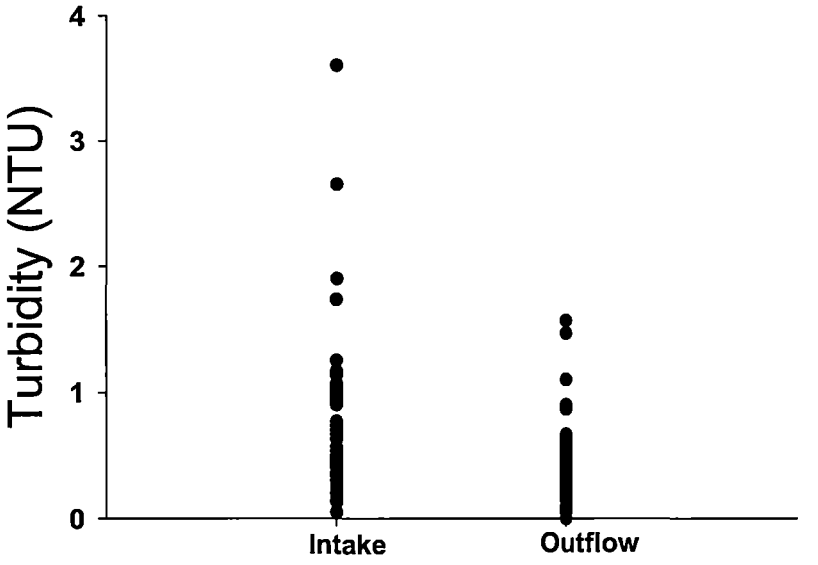

Figure 2.12: Nephelometric Turbidity Unit spreads over three 24 hour periods for the intake and outflow waters of Abalone farms Australia

Figure 2.13 shows that within the sedimentation ponds indicate that there is evidence of a diurnal trend in dissolved oxygen concentrations which is consistent over the three sampling times. The trend appears to be elevated dissolved concentrations during late afternoon to early evening (3-8pm) while concentrations were lowest at around 6-8am.

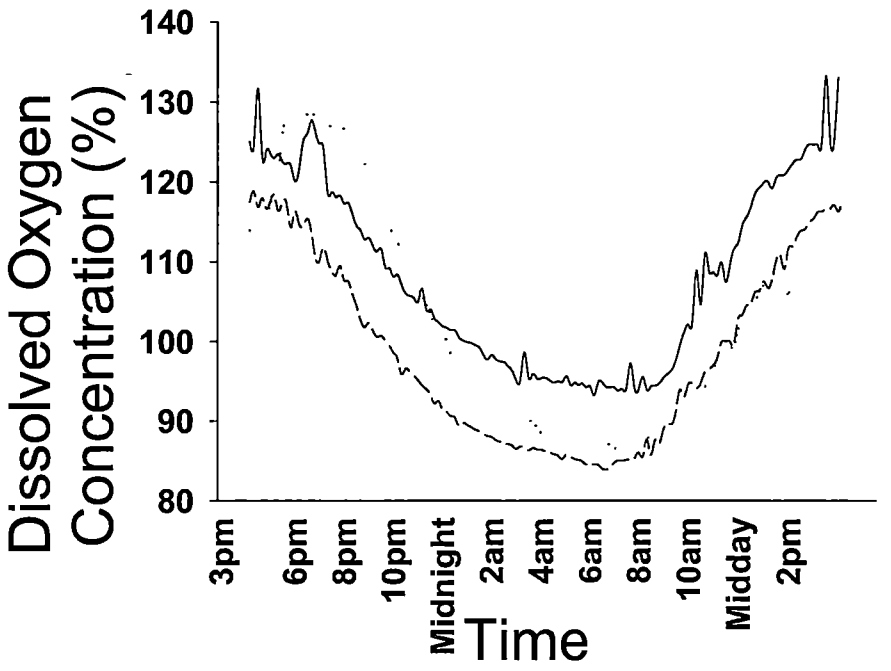

Figure 2.13: Dissolved Oxygen Concentrations (as percent saturation) over three 24 hour periods within the sedimentation pond waters of Abalone farms Australia

Figures 2.14 and 2.15 shows approximately 40-50% error in N and P associated with a single sample which is made either during the late afternoon (6-8pm) or around sunrise while relatively smaller errors were associated with sampling times around midnight and midday.

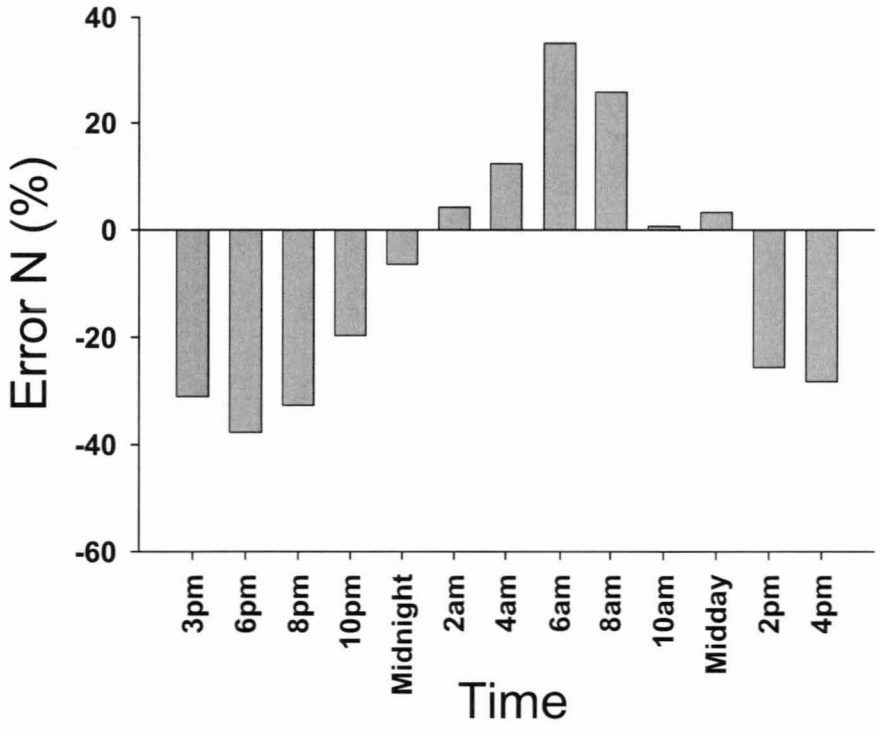

Figure 2.14: The error associated with estimating total daily effluent nitrogen loads from Abalone Farms Australia. Error = the cumulative load from 12 samplings in 24 hours (i.e. sampling every 2 hours) minus a single hour reading multiplied by 24 hours then divided by Cumulative load (24 hour sampling) x 100. Negative values indicate underestimation, positive values indicate over estimation.

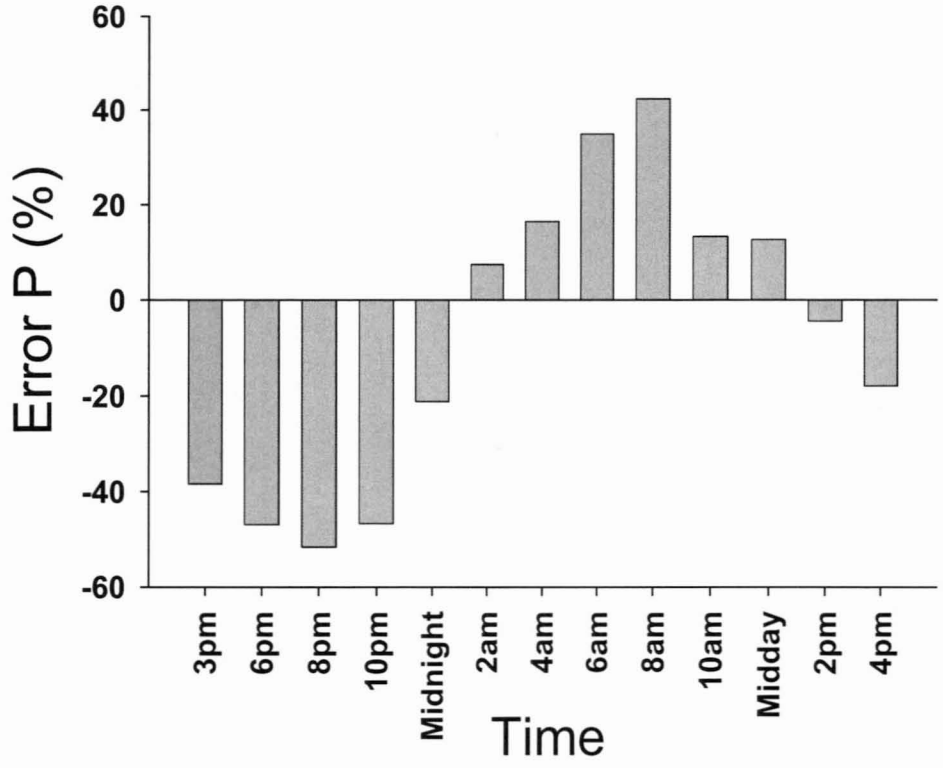

Figure 2.15: The error associated with estimating total daily effluent phosphate loads from Abalone Farms Australia. Error = the cumulative load from 12 samplings in 24 hours (i.e. sampling every 2 hours) minus a single hour reading multiplied by 24 hours then divided by Cumulative load (24 hour sampling) x 100. Negative values indicate underestimation, positive values indicate over estimation.

2.3.5 Discussion

The peak in dissolved nitrogen and phosphate concentrations at the outflow is distinct from any trend which may be occurring at the intake, and hence must be the result of on-farm sources. Given that the dissolved nitrogen is largely composed of

ammonium (i.e. 87% on average), we have considered ammonium and dissolved nitrogen mutually. Dissolved nitrogen is added to the water both through abalone excretion (ammonium) and also through other organisms within the farm sedimentation ponds and drains. The main areas of biological activity and therefore sources of ammonium are likely to be the culture tanks and the sedimentation ponds.

The tank compartments are likely to 'produce' some level of ammonium given that ammonium is the main excretory product of abalone (Barkai and Griffiths, 1987; Farías et al., 2003) however results of Evans and Langdon (2000) showed that 20 tonnes of abalone are only likely to produce 170g-N /day. After the abalone have consumed their formulated feed, a portion of the feed will be excreted as faeces and another portion excreted across the gills as ammonium. In addition to the direct excretion across the gills of the abalone, there may be some direct leaching of ammonium from formulated feed sources (Burford and Williams, 2001). It is unlikely leaching ammonium from the feed is influencing the peak exhibited at the outflow, as the residence time of the tanks and sedimentation ponds indicate that the peak in ammonium at 6am, would need to be exhibited at the tank outflow at 4-6 pm the previous night (i.e. 12-14 hour residence time). The only plausible possibility for the peak in ammonium in the tank compartment at 6pm is leaching of ammonium from feed because ingestion and subsequent excretion by the nocturnally feeding abalone (Shepherd, 1975) would not commence until after sunset (6:51pm) later in the day.

Dissolved nitrogen may also be 'produced' from the bacterial breakdown of organic matter within the sedimentation ponds and tanks and also the catabolism of amino acids and excretion by other organisms within the ponds. At AFA the uneaten feed

and faeces generated by the abalone are flushed from the tanks twice weekly and ultimately delivered to the sedimentation ponds. This particulate waste is likely to have a nitrogen component which if left within the sedimentation ponds will decompose and be remineralised as ammonium (Ackefors and Enell, 1994; Hargreaves, 1998; Holmer et al., 2003; McGhie et al., 2000; Smith, 1996a). Some of this ammonium is likely to be utilised within the sedimentation ponds by marine algae, hence reducing the dissolved load exported to the marine environment. The utilisation of ammonium by algae in the sedimentation pond, however, is unlikely to be consistent over a diurnal cycle primarily due to variation in irradiance and hence photosynthetic activity, nutrient uptake and growth by the algae. Therefore we can expect that during daylight hours, nutrients are utilised by the algae which may decrease outflow concentrations. As sunset and sunrise were 20:21 and 06:51 respectively on the day of sampling, and maximum ammonium concentration was recorded at 06:00 it may be that the lack of photosynthetic activity and nutrient uptake in the sedimentation ponds overnight results in greater outflow of ammonium. Variation in dissolved oxygen concentrations within the sedimentation pond showed reduced concentrations at 6-8am, and elevated concentrations during the mid to late afternoon (2-6pm) indicating greater photosynthesis during the day. Using this rationale, the diurnal changes in ammonium concentrations within the sedimentation pond are likely to be both a function of the uptake by algae and the production of ammonium by the abalone and other organisms within the farming system. Similar findings have been reported by a number of researchers studying aquaculture systems (Brune et al., 2003; Hargreaves, 1997).

Phosphate showed a peak at 8am on the day of sampling. This peak is likely to be driven primarily by the P losses associated with the feed entering the water as the peak in outflow concentration corresponds with feeding time (when the calculated residence time of the farm sedimentation pond and tanks are considered). Sales *et al.* (2003) found that rapid leaching of up to 30% of feed P from some abalone diets can occur within the first hour. The 50kg of feed which entered the water on the day of sampling contained 0.6% P and assuming Sales *et al.* (2003) figure of 30% lost; this equates to ~ 300g P. This 300 grams had the capacity to raise each litre of effluent (farm daily flow of 8.6 megalitres) by 2.5μm/L (i.e. ((300g÷ 14 grams per mole) *1,000,000(moles to μMoles) ÷ 8.6 megalitres). Therefore the P losses from the feed (assume similar results to Sales *et al.* (2003)) had more than enough capacity to drive the increases in P loading observed at the farm outflow and may be the driving force behind the observed diurnal trends.

It is also possible that the early morning peak in P seen at the outflow may be due to less primary production at night; however, if we assume that the diurnal reduction in dissolved nitrogen concentrations are due to algal uptake, then using the Redfield ratio the expected P uptake would only account for 8% of observed the P concentration. Therefore it is likely that the peak in P is mostly due to variation in production of P rather than variation in its consumption.

Concentrations of silicate showed no evidence of a strong diurnal pattern at the farm outflow which was clearly distinct from the inflow (as exhibited for both ammonia and phosphate). All concentrations of silicate were elevated at the farm outflow indicating that the farm was actually generating a silicate load to be exported to the

marine environment. The cement tanks which house the abalone may be the source of Si as unpublished data (Ho *et al.* PhD thesis) shows that newly finished cement tanks are capable of leaching up to 42grams of Si over a three day period. Other possible sources include the artificial feed leaching and sedimentation pond waste remineralisation.

Relative to the outfall the intake water showed a high degree of variability in turbidity. This is likely due to coastal wave action combined with the build up of seaweed and particulate matter in the granite intake pit. As water flows through the farm, the affect of the sedimentation ponds is to reduce water column particulates derived from the intake waters, wind (terrestrial input into sedimentation ponds) or on-farm practices (e.g. cleaning).

It can be seen that ammonium and phosphate represent the two nutrients which are likely to be 'produced' within the farm at relatively high concentrations and hence the dynamics of their nutrient flux is of importance to monitoring program sampling protocols. Specifically the highest concentrations of ammonium at the point of discharge would be likely recorded at sunrise, and for phosphate the peak is likely determined by the residence time of the grow-out tanks and sedimentation ponds (in this case 12-15 hours post feeding). The results of this experiment also indicate that the time for sampling of nutrients at the farm outflow is probably best around midday, when the error associated with extrapolation of single measurements of P and N to get a daily load is relatively low for both nutrients. There was a reasonably large variation in the amplitude of the diurnal flux of nutrients (ammonium and phosphate), and the size of this variation is hypothesised to change through seasons given the strong influence of photosynthesis (and hence light regimes) on the nutrient concentrations.

The information provided from this trial is likely to assist government agencies around Australia who currently have monitoring programs in place for abalone farms. Information such as the most appropriate sampling time for the capture of the peak in nutrient concentrations and also the diurnal variability in concentrations of nutrients are of importance to the sampling and interpretation of the monitoring program results. Given that for most monitoring programs farmers are required to take their own water quality samples and submit them for analysis and that there is no set protocol for sampling (i.e. time, water flow rates, cleaning versus not cleaning, where the sampling should take place, what depth of water) there may be considerable variation in the sampling conditions between sampling periods (within a monitoring program). For example, if a farm consistently increased the total amount of nitrogen measured in a series of monthly samples, this could be a function the variation between sampling times (i.e. time of day) or the farm could be increasing its export of nitrogen. The results here highlight the importance of the consistency in water sampling between sampling periods within a monitoring program and indicate future work in this area may aim to develop a suitable protocol or industry standard for water sampling.

Additional research is also needed on the change in the diurnal rhythms through seasons and the level of representation that these results have relative to other abalone farms around Australia.

2.4 Chapter Discussion and Conclusions

The results of the chapter show that the main sources of nutrient production within the abalone farming system are associated with the formulated feed but primarily the processes driving variation seem to be occurring within the settlement ponds. Once the abalone formulated feed enters the culture tanks, there is a limited number of pathways its constituents can take. It can leach into the water column, it can be consumed by the abalone and then be excreted (either dissolved or particulate) or it can remain uneaten. If we consider the possibilities for the fate of the formulated feed there is limited leaching of N and relatively little dissolved excretion of N by the abalone suggesting that the tank particulate waste is one of the main sources of nutrient pools within the system other than the abalone themselves. That this particulate waste then is retained within the abalone farm, suggests that this is likely the largest labile nutrient pool within the farming system. This information combined with the clear reductions in concentration of the formulated feed/particulate waste indicates that the diurnal variations in the concentrations of nutrients is driven by the processes related to the particulate waste (the majority of this resides within the settlement pond as tanks are simply a transient harbour of particulate waste as they are cleaned twice per week). This is further supported by the dynamic nature of the settlement pond where the strong presence of macrophytes and filamentous algae were observed over the course of the study. Hence given the particulate waste and the likely remineralisation of dissolved nutrients (Boyd, 1992; Burford, 1997; Burford and Longmore, 2001; Burford and Williams, 2001; Hargreaves, 1998) it seems that the evidence shows processes of photosynthesis are

occurring within the ponds. Thus the settlement ponds appear to be exhibiting the classical patterns of water quality (particularly nutrients and Dissolved Oxygen) that occur within aquaculture grow out ponds (Burford, 1997).

CHAPTER 3: Characterisation of abalone farm effluent

3.1 Introduction

Worldwide abalone farming has increased by 1200% in the 15 year period to 2002 (Gordon and Cook, 2004). In Australia abalone aquaculture has increased steadily from 3 tonne in 1998 to approximately 66 tonne in 2002 (Fleming, 2003). This expansion is part of the dramatic increase in competition for marine resources that is causing concern among some government authorities and environmental groups. Issues surrounding the long term environmental sustainability of the industry have been raised and require addressing to ensure the protection of both the environment and eventually the industry. Possible means of addressing the issue of sustainability are the processes of Environmental Management Systems (EMS) and Ecologically Sustainable Development (ESD). Both systems use research to assess the risks of various aspects of farming and develop best management practices (Seafood Services Australia Ltd, 2004). At present there is a paucity of information as to the effects of land based abalone farming on the marine environment receiving the discharge water. This lack of information is required for the progression of the EMS and ESD processes. Further with the growth of the abalone industry, unsustainable practices may be allowed to develop causing future problems with the environment and also the industry acceptance of the EMS and ESD processes. For the abalone industry to move ahead with the concepts of environmental sustainability, research into the environmental impact of abalone farming needs to occur.

ESD and EMS are systems of assessment which can be implemented into individual operations or on an industry wide basis. They may also vary from individual

operation to operation in terms of the intensity of assessment and regulation. The basic principles involving ESD and EMS is that risk is assessed on an individual basis providing framework to assess the risk yet allowing flexibility to tailor for individual situations. For example, two abalone farms may use the same ESD or EMS template to determine the likelihood of disease translocation, however the two different farms may have drastically results as to the measures taken to reduce disease translocation risks. It is these risk assessments that then form the basis of the operational guidelines for the farming operation and give consistency, structure and a methodical approach to the daily operations of an abalone farm. These processes of ESD and EMS are therefore likely to provide insight to abalone farmers as to the limitations of the marine environment which their business relies on, as well as the means to ensure that they have consistent, well calculated and documented practises.

While there has been no research published that examines the environmental performance of abalone farms, a number of studies have examined the environmental impacts of other land based aquaculture facilities. In particular nutrient dynamics have been studied on prawn (Abdul Wahab et al., 2003; Funge-Smith and Briggs, 1998; Jackson et al., 2003a; Shahidul Islam et al., 2004) and freshwater finfish facilities (Johansson and Nordvarg, 2002; Knosche and Schreckenbach, 2000; Krom and Neori, 1989; Michael Jr, 2003). Of a similar nature to abalone farming is prawn culture yet fundamental differences exist between the two types of culture which affect the particulate and nutrient dynamics of the system and hence the potential for environmental impacts. For example, abalone systems tend to be housed in cement or plastic tanks while prawn culture tends to occur in earthen ponds. In terms of particulates, feed input only

accounts for 4-7% of the total particulate budget in shrimp farms (Funge-Smith and Briggs, 1998). By far the biggest input of particulates was erosion of pond soil which accounted for 88-93% (Funge-Smith and Briggs, 1998). In an abalone system with cement tanks, it is likely that feed input will account for the majority of the particulates given the culture water comes into contact with cement surfaces only (i.e. not exposed to underlying soil as in prawn culture). There are also a number of differences in terms of dissolved effluent characteristics between abalone farms and prawn farms. Abalone farms tend to have high water flow relative to prawn farms and therefore the effluent can be expected to be more dilute. Additionally green water culture exists in prawn farms where blooms of algae are promoted whereas this is not the case for abalone farms where clear water is required. These differences combined with the siting of abalone farms (i.e. usually in high energy coastlines) compared with prawn farms (i.e. sheltered low energy coastline) causes there to be some distinct differences in terms of the likely environmental impacts of abalone farming.

Despite these differences it is likely, however, that some of the nitrogen processes within the culture systems will be similar between prawn and abalone culture. Typically the intensive culture of aquatic species results in elevated concentrations of ammonium that has been excreted as a by product of animal metabolism (Pearson and Black, 2001). The fate of this ammonium is generally biological uptake by phytoplankton or macrophytes, or bacterially mediated nitrification - which is the conversion of ammonium to the oxidised form of nitrite and then to nitrate (Hargreaves, 1998). The particulate organic waste generated by the consumption and excretion of artificial or natural diets will eventually result in ammonium efflux from the sediments into the water column

(Christensen et al., 2000). While much of the organic waste in an abalone system is flushed into sedimentation ponds, in prawn culture it occurs within the culture ponds in concentrated areas (generally the centre due to water circulation/dynamics within a pond).

The vast majority of the growth in abalone aquaculture has been in land based farming systems which have point source discharges (Hone and Maguire, 1996). A single point discharge presents an easy and relatively cost effective means of monitoring (i.e. the difference between the farm inflow and farm outflow = a measure of environmental performance). While this may provide a low cost strategy for monitoring environmental performance some vital pieces of information remain unknown. For example it is not known how the discharge of nutrients from an abalone farming system fluctuates (with respect to nutrients and particulates) on any temporal scale. The effects of different environmental parameters (such as rain and swell conditions) on the results obtained is also not known. Thus it is possible that temporal variability in nutrient discharge combined with limited sampling may cause inaccuracy in the conclusions derived from the data collected within monitoring programs. On a spatial scale, the relative contributions of different sections of the farm (i.e. compartments = the tanks, drains, sedimentation pond(s)) towards the net total farm output is unknown. Such background information is important for improvement of effluent water quality and greater validity of the results generated from monitoring programs.

This study characterised the role of various abalone farm compartments in the context of the farm's total environmental performance. The study site was Abalone Farms Australia (AFA), Bicheno on the East coast of Tasmania, Australia. An intensive

monitoring program which monitors variables of water quality; variables of the system such as feed rate and biomass, and sampling conditions such as rainfall and cleaning was used to substantiate the minimal monitoring regime currently in place within Australia. This study supplements existing monitoring programs and determine the key variables for efficient monitoring.

3.2 Materials and Methods

An 18 month monitoring program was implemented to determine the contribution of various farm compartments towards the net total environmental performance (inflow water quality-farm outflow water quality) of Abalone Farms Australia. In this study environmental performance was assessed using Total Suspended Solids (TSS) and the output of nutrients ammonium, nitrate, nitrite, phosphate and silicate. The compartments measured within the farm are the tank systems, the main drain and the sedimentation pond system. To characterise these compartments the inflowing water and the outflowing water to and from each compartment, was sampled on a monthly basis. If inflow > farm outflow the compartment was classified as net consumer.

3.2.1 Farm information

Abalone Farms Australia is a land based abalone farm on the East coast of Tasmania. Since June 2002, the farm has been increasing production of abalone with the commissioning of approximately 150 new cement tanks over the duration of the sampling period. At the cessation of the sampling period (September 2004) the total abalone biomass on the farm was between 18-20 tonnes. Both greenlip (*Haliotis laevigata*) and

hybrid (*Haliotis sp.*) abalone cultured within concrete tanks (6.5 x 2.5m). The sedimentation pond system has three lined 1000m^2 ponds with a total capacity of approximately 5 megalitres and a residence time of 12.5 hours. During the 18 month period of this study only a single sedimentation pond had been commissioned with a capacity of 1.5 megalitres and residence time of 5 hours (Fig. 3.1).

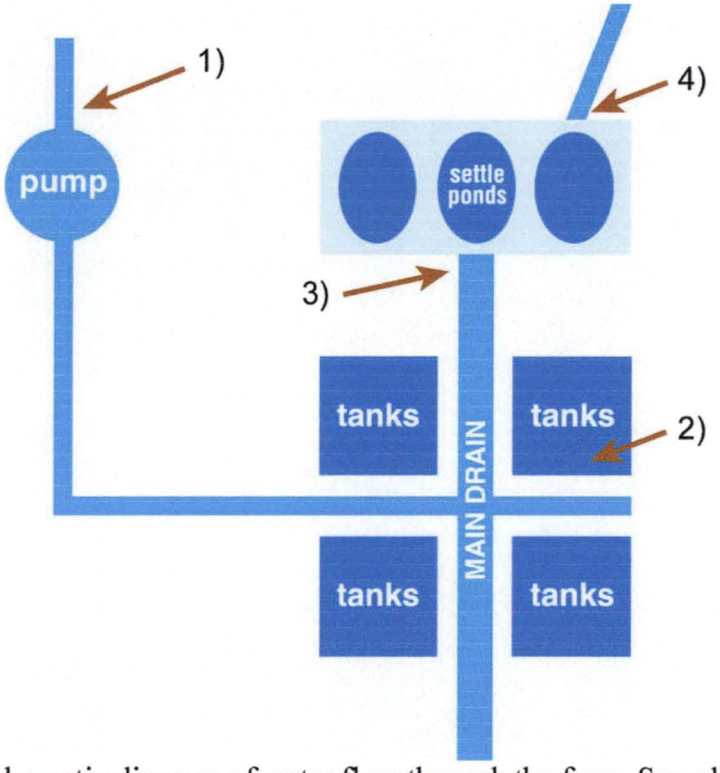

Figure 3.1: Schematic diagram of water flow through the farm. Sampling points are listed in order of sequence (1-4): 1 = farm inflow, 2 = tank outflow, 3 = sedimentation pond inflow, 4 = sedimentation pond outflow.

3.2.2 Nutrients

When sampling below the surface a Niskin water sampler (General Oceanics model #1010) was used. The Niskin was rinsed three times in the water to be sampled,

shaken, decanted and then sampling occurred. Labelled 10ml polypropylene sterile tubes (Sarstedt, catalogue # 60.9921.819) were filled with the sample water. Where use of a Niskin was not possible, tubes were held under the water flow (i.e. at the tank outlets). All samples were kept out of direct sunlight and transferred immediately to a contaminant free clean freezer and stored at -20 °C. The samples were analysed for nitrite, nitrate, phosphate and silicate on a Technicon® AAII autoanalyser as outlined in (Plaschke, 1999), Ammonium analysis was conducted according to the method outlined in (Watson et al., 2004).

3.2.3 Sampling compartments

To characterise the various compartments, sampling was conducted at the inflow, tank outflow, sedimentation pond inflow, and sedimentation pond outflow (Fig. 3.1). The farm inflow is a 40-50 m^3 rocky reservoir holding ~ 40,000L which connects to the ocean through a narrow channel. Within this reservoir are submersible pumps which supply the farm with seawater. Samples were taken using the Niskin in the reservoir from a depth of about 5.5m and as close to the submersible pump intakes as practically possible.

Tank outflow samples were taken directly from the continuous flow out of an 80mm PVC pipe which collects the outflows from 52 tanks. These 52 growout tanks were initially stocked with animals ranging from between 5-20mm and over the period sampled the total biomass in these 52 tanks increased from approximately 350kg to 6000kg. The 52 tanks represent a subsample of the farm's grow-out tanks (total = 182 grow-out tanks which held approximately 95% of the total farm biomass, 26 nursery tanks holding approximately 5% of the total farm biomass). Results from the outflow of

52 tanks were scaled up to the whole farm and adjusted to account for the dilution of grow-out tank effluent (i.e. 182 tanks make up 87.5% of total water flow through farm) by the nursery tank effluent (nursery tank effluent was assumed to be zero nutrient concentration). The sedimentation pond inflow was sampled at the junction of the main drain and the sedimentation pond. Typically the water depth at this point was approximately 20cm and sampling was conducted as close to mid water column as possible. Sampling was always conducted with the containers filling upstream of human hands to avoid sample contamination.

The sedimentation pond outflow water was collected at a point after the water had left the sedimentation pond but before the water had been returned to the ocean. Similar depths and protocols to sampling at the sedimentation pond inflow sampling were employed. All samples were filtered prior to storage as described below (25mm, Whatman GF/F nominal pore size 0.7μm).

3.2.4 TSS+ Particulate Organic Matter (POM)

Modifications to standard method 2540-D (Franson, 1989) were used to determine TSS. Pre washed, ashed and pre weighed (to ± 0.00001g) GF/F filter papers (Whatman 25mm, 0.7μm nominal pore size) were used to filter water samples. Samples for TSS were collected in 200ml polypropylene bottles (rinsed three times with sample) and subsequently filtered using GF/F filters held in a Swinex™ syringe filter holder. The sample volumes filtered were recorded. Ten mL of air was pushed through the filter to ensure all the seawater was filtered. The resulting filter was dried at 105°C for 48 hours on a filter holding tray (aluminium foil lined), reweighed and further checked for constant

weight after another 12 hours of drying. POM was determined according to Standards Methods 2540-E (Franson, 1989) with all weight measurements taken using a five decimal point balance (Mettler AE240).

3.2.5 Silicate and cement tank experiment

Three newly poured tanks were flushed and filled with ambient seawater and left static for a period of 3 days (time usually left to 'soak' before draining and adding animals) with aeration. Samples of the initial ambient incoming seawater were taken and no animals were in the tanks at the time of the experiment. After three days the water flow was turned on to a standardised rate (AFA standard flow rates) of 25L/min/tank and samples of the tank outflow water were then taken in triplicate and stored at -20°C. These samples were analysed for nutrients as above. According to Bureau of Meteorology data no rain fell over the 3 day duration of this experiment.

3.3 Results

3.3.1 Dissolved inorganic nutrients

3.3.1.1 Ammonium

The total farm concentration of ammonium showed an increase over time eventually reaching seven times the concentrations in the farm inflow water (Fig. 3.2). The tanks and sedimentation ponds were the primary drivers behind the total farm ammonium loads exhibited. Counteracting these positive loads was the drain acting as a

sink for ammonium (on average removing 40% of the total farm ammonium concentration).

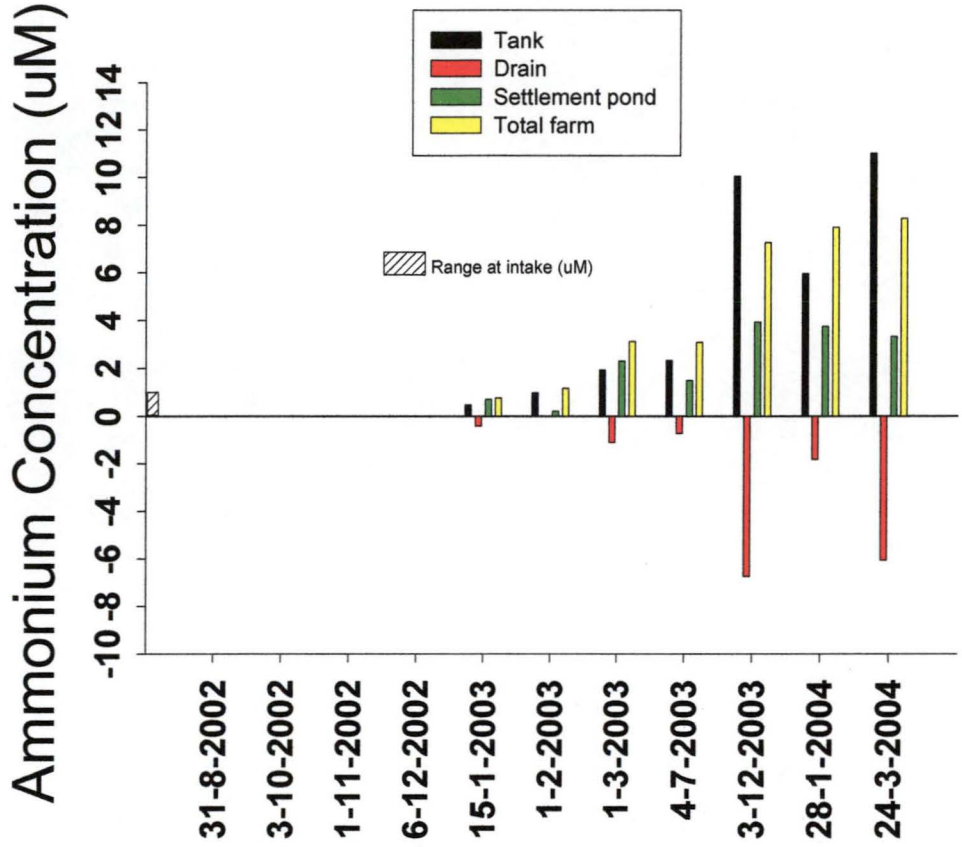

Figure 3.2: Ammonium production or consumption concentrations for different compartments (Tanks, drains, Sedimentation pond and total farm). Positive values indicate production, negative values indicate consumption. Each value is the result of the subtraction of the mean of three samples at the inflow and outflow of that compartment.

There was a significant relationship between daily feed rates and total farm ammonium + NO_x (NO_x = nitrite + nitrate) export as measured over the 12 months of the study (Fig. 3.3). This relationship described 54.5% of the variability within the data set.

Closer analysis reveals an outlier in terms of $NH_4 + NO_x$ (this outlier was confirmed based on anomalous salinity of the farm outflow sample) and by omitting this point the relationship explains 98% of the variation in $NH_4 + NO_x$ concentration. The presence of an outlier based on salinity (this sample was 23ppt when all other samples were >34ppt) implies a possible rainfall and ammonium interaction; however no significant relationship was found between rainfall and ammonium (Pearsons correlation R = 0.588, P= 0.125, n=8) or salinity and ammonium (Pearsons correlation R = 0.588, P= 0.125, n=8). All other samples were tested and ranged between 34 and 35.5ppt.

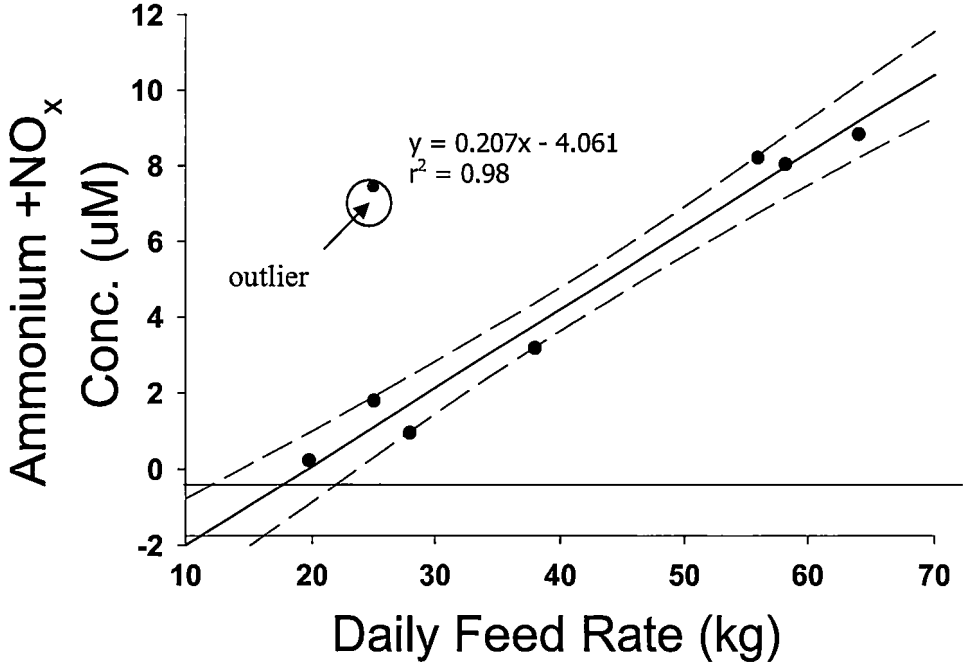

Figure 3.3: Daily feed rate and net total farm ammonium +NOx (nitrite+nitrate) concentration (concentration calculated by the mean of three farm outflow - mean of three farm inflow concentrations). Solid black line is linear regression omitting the outlier; dashed lines are 95% confidence intervals for the regression line.

3.3.1.2 Nitrate

Nitrate concentrations in the various compartments (Fig. 3.4) showed no discernable temporal patterns that were attributable to any of the recorded variables. On most occasions the total farm was a sink for nitrate and all compartments on some occasion contributed to this overall trend. Interestingly from Dec 2003 onwards the farm became a producer of nitrate and the tanks were the main factor influencing this shift. All the concentrations recorded for all compartments over the sampling period were within the ambient range recorded at the farm inflow.

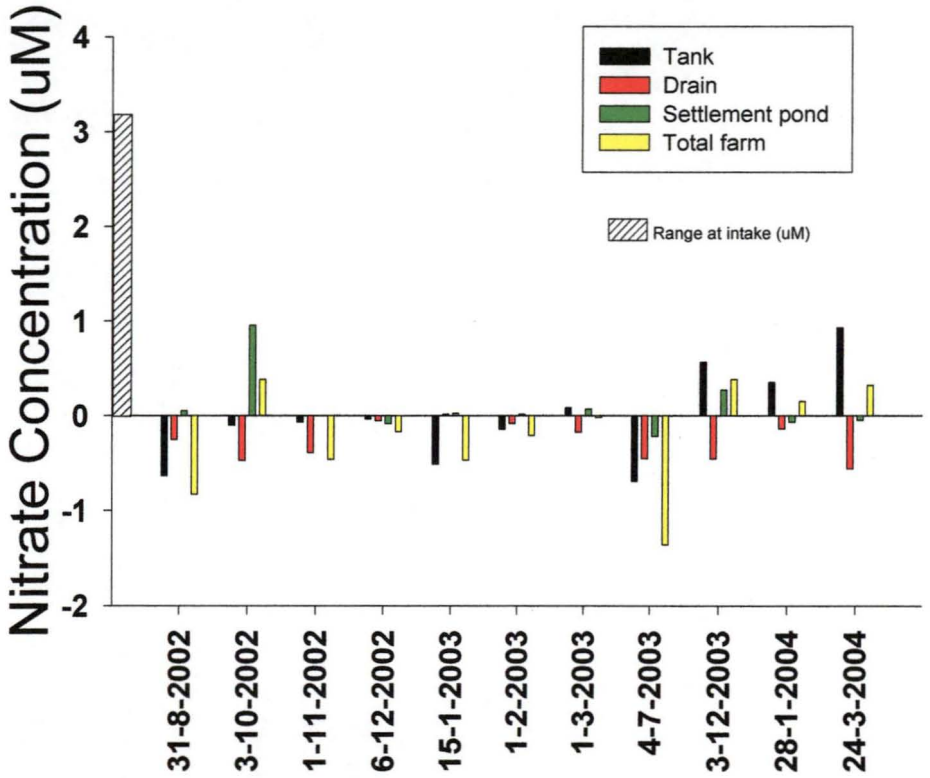

Figure 3.4: Nitrate production or consumption concentrations for different compartments (Tanks, drains, Sedimentation pond and total farm). Positive values indicate production, negative values indicate consumption. Each value is the result of the subtraction of the mean of three samples at the inflow and outflow of that compartment.

3.3.1.3 Nitrite

Total farm concentrations of nitrite increased over the period sampled (Fig. 3.5). The tanks and sedimentation pond were the major contributing compartments with the drain on most occasions consuming nitrite. Total farm ammonium concentration and nitrite concentration correlated well (Pearsons R = 0.805, P= 0.016, n=8) showing a very similar temporal pattern.

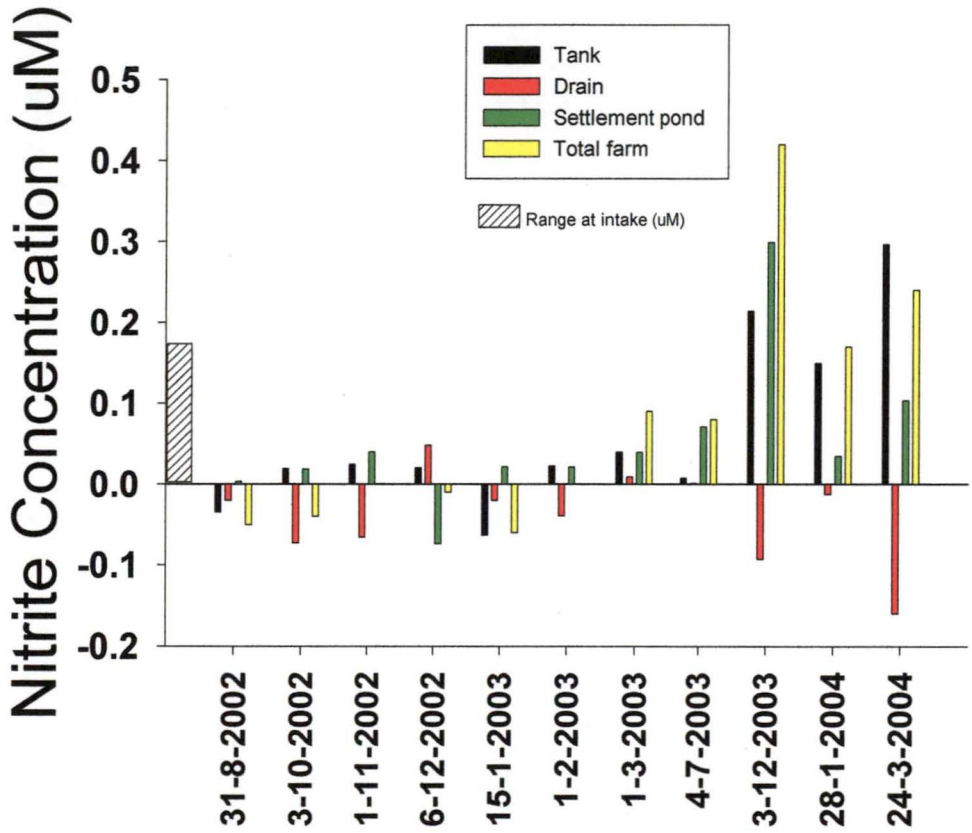

Figure 3.5: Nitrite production or consumption for different compartments (Tanks, drains, Sedimentation pond and total farm). Positive values indicate production, negative values indicate consumption. Each value is the result of the subtraction of the mean of three samples at the inflow and outflow of that compartment.

3.3.1.4 Phosphate

Total farm concentrations of dissolved phosphate increased throughout the sampling period. The tanks; and on some occasions the sedimentation pond, were supplying the increase in total farm concentration (Fig. 3.6).

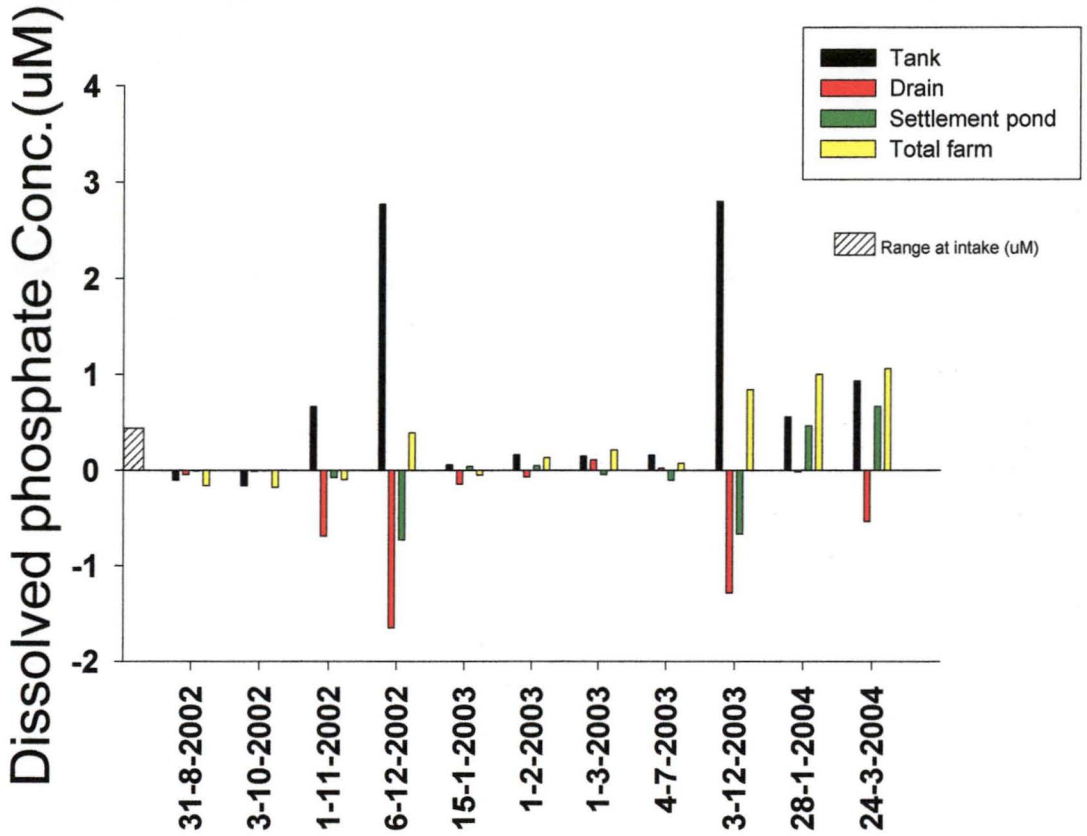

Figure 3.6: Dissolved phosphate production or consumption for different compartments (Tanks, drains, Sedimentation pond and total farm). Positive values indicate production, negative values indicate consumption. Each value is the result of the subtraction of the mean of three samples at the inflow and outflow of that compartment.

The sedimentation pond showed no significant net production of dissolved phosphate until Jan 2004. The drain was consuming dissolved phosphate counteracting its production in the tanks and proportionately the more dissolved phosphate the tanks produced the more dissolved phosphate was consumed in the drain. There appeared to be a seasonal effect of dissolved phosphate production in the tanks and consumption in the sedimentation pond with greater rates of consumption and production occurring in the late spring early summer months during both 2002 and 2003 (Fig. 3.6). Investigation into the sources of P within the formulated feed found that the P supplement constitutes approx 0.2% of the 0.68% or 29% of the total P content of the feed (Scanlon, Personal Communication)

There was a significant relationship between feed rates and total farm dissolved phosphate concentration with 80% of the variability being explained by the relationship (Fig. 3.7). An outlier was omitted from the regression based upon the lower salinity (23ppt) of the sample (i.e. as indicated for ammonium).

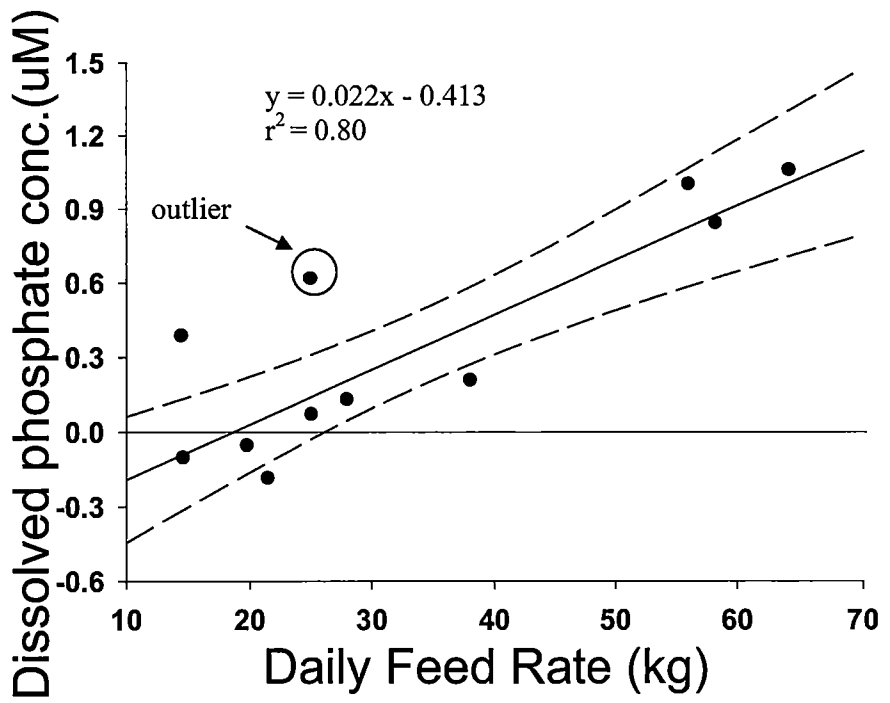

Figure 3.7: Daily feed rate and net total farm dissolved phosphate concentration (concentration calculated by the mean of three farm outflow - mean of three farm inflow concentrations). Solid black line is linear regression omitting the outlier, dashed lines are 95% confidence intervals for the regression line

3.3.1.5 Silicate

The farm always produced silicate over the period sampled (Fig. 3.8). The majority of this silicate was produced from the tanks and sedimentation ponds (average 100% and 23% of total farm silicate respectively), while the drains consumed an average of approximately 24% of the total farm Si. During every sampling period the tanks produced approximately 1μM Si with relatively little variation when compared with other compartments. In contrast within the sedimentation pond, production and consumption

varied with consumption yielding changes in the concentration of silicate ranging from -0.5 μM to production changing concentrations by +1.5 μM. The sedimentation pond silicate production correlated well with total farm silicate production (Pearsons R = 0.983, $P<0.001$, $n=12$) levels indicating that the trends exhibited by the total farm may be a function of the silicate production in the sedimentation pond. Sedimentation pond silicate concentrations were correlated with rainfall (Pearsons R = 0.646, $P=0.023$, $n=12$). Typically the concentrations produced from all compartments were within the range of ambient seawater (i.e. concentrations at the farm intake). The artificial feed contained relatively low amounts of silicate (130mg/kg).

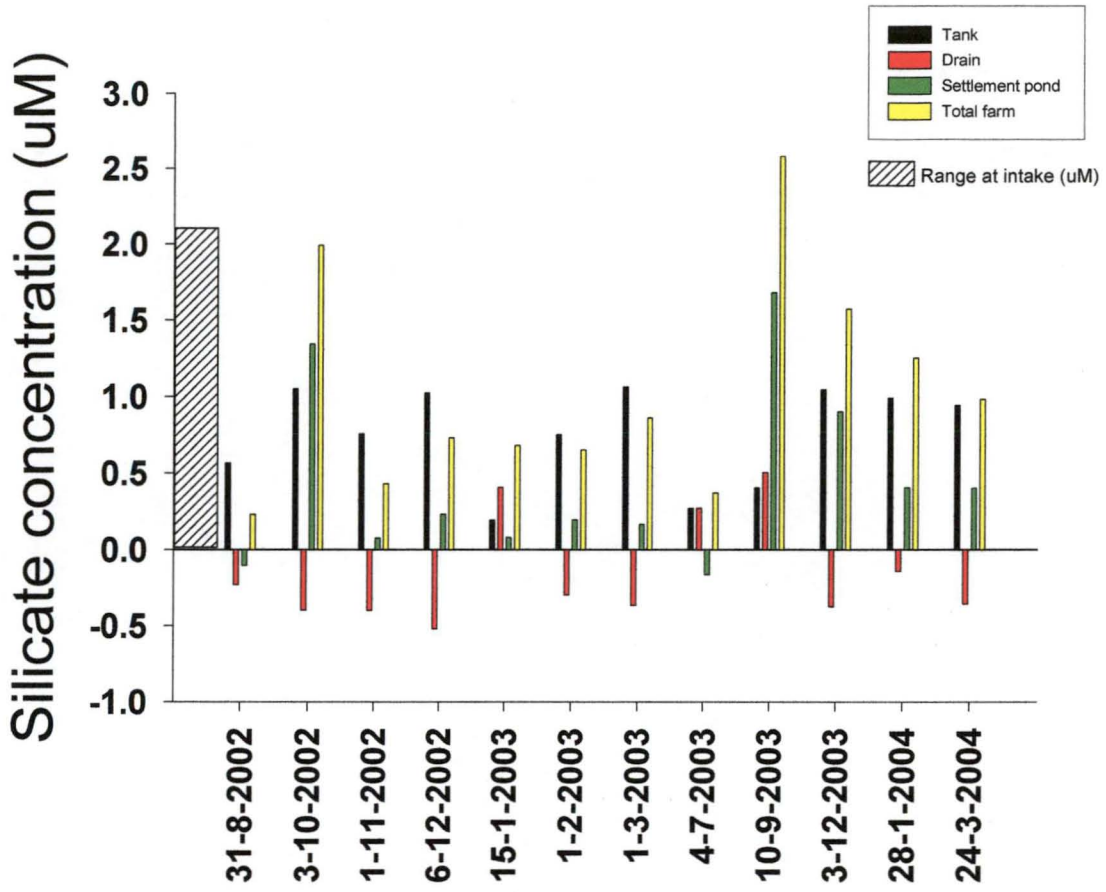

Figure 3.8: Silicate production/consumption concentrations for different compartments (Tanks, drains, Sedimentation pond and total farm). Positive values indicate production, negative values indicate consumption. Each value is the result of the subtraction of the mean of three samples at the inflow and outflow of that compartment.

3.3.1.5.1 SILICATE AND EMPTY TANK EXPERIMENT

Newly constructed tanks had high concentrations of effluent silicate in the order of 217-340 µmol/L (mean ± SE = 276 ± 36.1). These tanks here were clearly a source of Si given the much lower silicate concentrations of the ambient seawater that the tanks

were initially filled with. These concentrations translate into the capacity of each tank being able to raise Si concentrations by between 0.08-0.13µmol/L above ambient seawater over three days (Appendix) or the leaching of 17.5mM of Si per square metre of tank surface per day.

3.3.2 Particulate Waste

3.3.2.1 TSS and POM

Results indicate that in the majority of cases there was no net production of TSS by the abalone farm (Fig. 3.9). On one occasion only there was a total farm net production of TSS ($P<0.001$, df= 5, F= 452.9).

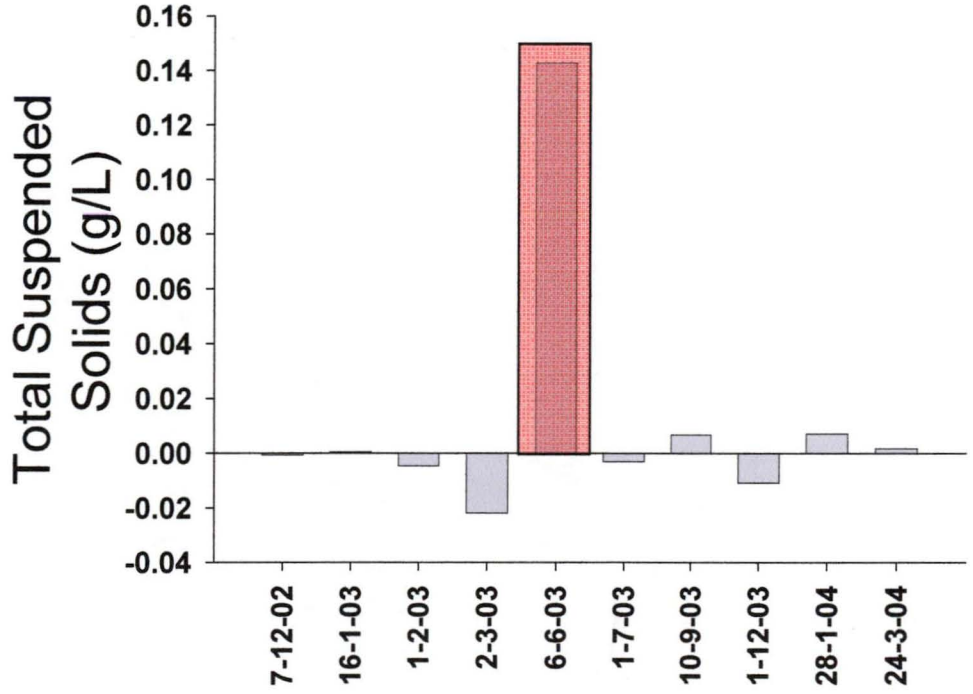

Figure 3.9: Mean farm outflow (n=3) - mean farm inflow (N=3) TSS concentration (farm outflow and farm inflow values were significantly different if highlighted red)

As a fraction of TSS, the particulate organic matter consumption or production by the farm ranged between consumption of approximately 1% to the production of 10% (Fig. 3.10).

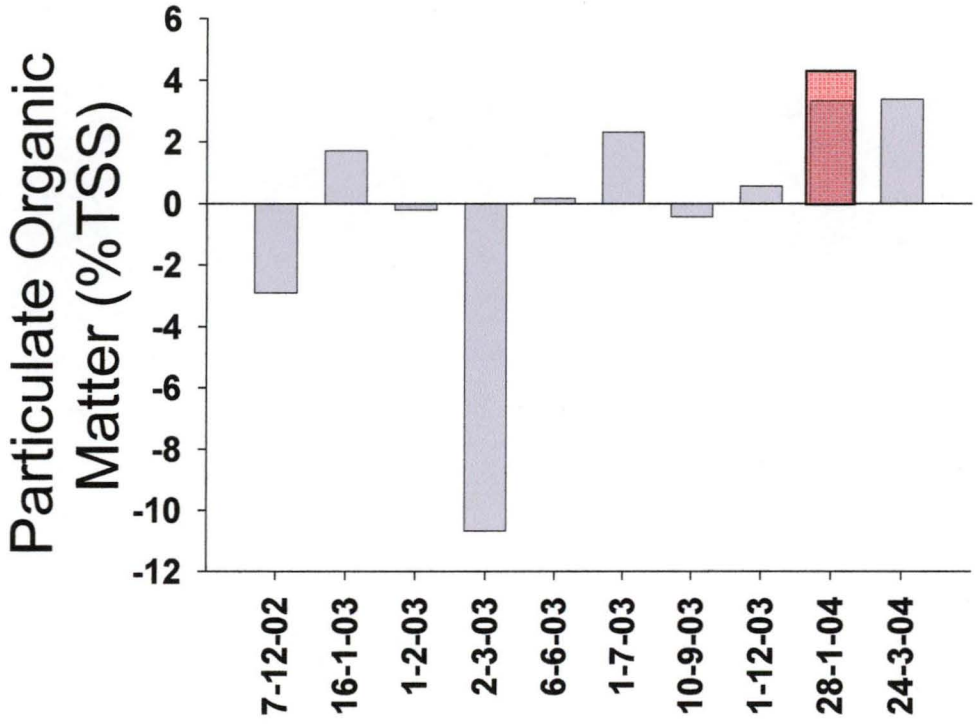

Figure 3.10: Mean farm outflow (n=3) - mean farm inflow (n=3) of Particulate Organic Matter concentration as a percentage of Total Suspended Solids (farm outflow and farm inflow values were significantly different if highlighted in red)

3.3.3 Farm nitrogen budget

A simple farm nitrogen budget (summed over all compartments) was constructed for AFA with an approximate abalone biomass of 18-20 tonnes (Fig. 3.11). The

particulate pool made up of faeces, uneaten feed and particulates from the farm inflow seawater, contained the largest pool of resulting nitrogen (~ 45%), followed by the abalone harvest and then dissolved nitrogen. There was no export of particulate nitrogen observed.

Abalone harvest kg-N/year

Nitrogen budget conducted suggests 37.5% of N fed to abalone will be retained as meat tissue (Neori et al., 2000). Therefore as feed N content is 1225.3 kg the abalone harvest will approximately be 460 kg-N.

Dissolved Nitrogen pool (released from farm) kg-N/year =

Kg-N/year = 8.8μmol/L x daily water flow rate (L) x N molecular wt x 365 days/1,000,000,000 (i.e. conversion factor from μg to kg)

=8.8μm x 8,640,000 x 14 x 365/1,000,000,000

=388.5kg-N/year

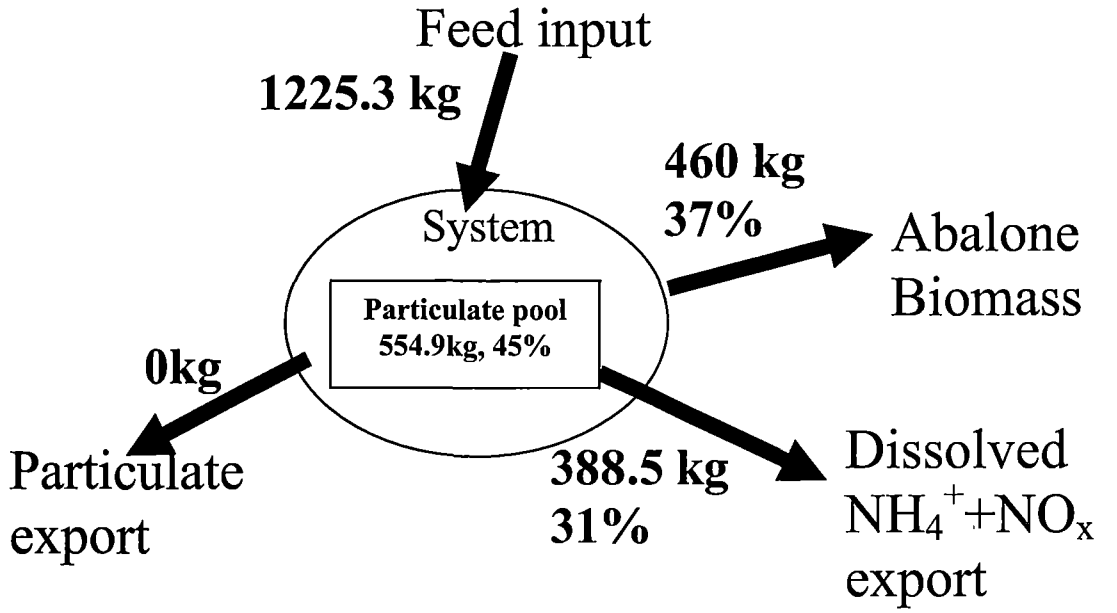

Figure 3.11: Nitrogen budget for Abalone Farms Australia based upon an annual feed usage for a farm holding approximately 20 tonnes of biomass.

3.4 Discussion

Total farm ammonium concentrations increased over time relative to the ambient farm inflow concentrations of ammonium. Given that our marine ecosystems are nitrogen limited (Thompson and Hosja, 1996) this suggests that ammonium represents the primary environmental concern of all nutrients discharged. As expected the tanks produced the highest ammonium concentrations and were the dominant component of the farm ammonium budget. This is expected as ammonium is an end product of protein metabolism and subsequent excretory product of abalone (Barkai and Griffiths, 1987;

Farías et al., 2003). Barkai (1987) found ammonium excretion rates can be as much as 1.6μm/h/animal (100g wet weight) at 19°C for the South African abalone.

In the later months of 2003 when ammonium production began to increase, the production of nitrite and nitrate in the tanks also increased. Within the highly aerated tank system nitrification (conversion of ammonium to nitrate) is most likely occurring where nitrite is the intermediate step. That the concentration of nitrite and nitrate is significantly lower than ammonium (only 5% conversion) suggests that either the rate of nitrification is lower than the rate of ammonium production or there is a sink of nitrite/nitrate. A sink of nitrite is unlikely given its toxic nature (Jensen, 2003) and the high energy requirement for assimilation by algae relative to ammonium (Lobban and Harrison, 1994). A sink of nitrate would imply some level of uptake by marine algae, or perhaps a microbiological process such as denitrification. Phytoplankton will preferentially use ammonium (Eppley et al., 1969; McCarthy, 1981; Syrett, 1981) over nitrate and nitrite due to the lower energy expenditure required to assimilate these forms of nitrogen into amino acids (Lobban and Harrison, 1994). Given the relatively large amounts of ammonium, nitrate is unlikely to be utilised by phytoplankton under these conditions.

Denitrification is similarly unlikely. As denitrification commonly occurs in anoxic conditions (Hargreaves, 1998) it is unlikely that with the high levels of aeration and movement of abalone on the tank floors that any anoxic zones of significance exist within the tank environment.

It is likely that the rate of ammonium production is greater than the nitrification rate. This is perhaps due to the tanks having a limited opportunity to establish a biofilm of *Nitrosomonas/Nitrobacter* colonies due to the relatively small surface area of the tanks

(i.e. lack of sediment), grazing of biofilms by the abalone (reducing establishment of colonies), and the organic content of the waste, which inhibits the proliferation of nitrifying bacteria (Yamagiwa et al., 1998). This is also coupled with the short residence time of the tanks (6.5 hrs) and the periodic scrubbing of the tanks when cleaning (i.e. two times per week). Low nitrite and high ammonium was also found in channel catfish ponds (Tucker and Van Der Ploeg, 1993) and at a Hawaiian aquaculture facility (Ziemann et al., 1990).

The drains were consuming ammonium, nitrate and nitrite during the majority of sampling times. There was comparatively much more ammonium being consumed within the drains than nitrate and nitrite. This appears to be a function of the lower concentrations flowing from the tanks rather than an actual difference in efficiency of the drains to convert the different N species. On average the drains reduced 43% and 47% of the ammonium and nitrite produced from the tanks, respectively. As nitrate showed little production from the tanks (consumption was more prevalent) an efficiency value was not calculated. This consumption of inorganic nitrogen is likely to be due to biological uptake from phytoplankton, macro algae or bacteria. The shallow, highly aerated drains have good water flow and light which make them suitable for photoautotrophs which for most times throughout the year populated the drains and tanks. Commonly populations of benthic encrusting microalgae, brown filamentous macroalgae (Fig. 3.12) and small populations of *Ulva* sp. were observed in the drains and tanks.

Together with various other presumably present microalgal and bacterial species, these micro and macroalgae consume dissolved inorganic nitrogen.

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Figure 3.12: Photograph of AFA main drain showing populations of brown and green macroalgae.

The sedimentation pond produced ammonium, nitrite and occasionally nitrate. The source of this nitrogen is likely to be the tank waste that is high in organic matter. In the sedimentation pond undigested and uneaten food was likely to be broken down and consumed by bacteria and other organisms within the sediments. The utilisation of these

proteins results in the remineralisation of ammonium as a by product (Hargreaves, 1998). Results from tracer studies (Nixon and Pilson, 1983) indicate that the nitrogen associated with organic matter is likely to have a half life of 1-2 weeks at 16°C with 90-95% remineralised into the water column. Observations of the sedimentation pond over time indicated that there was an initial period of approximately 3months (August -October 2003) where the level of the particulates accumulated followed by a period of consumption of particulates by various organisms within the pond (November onwards). It appears that the sedimentation pond 'matured' during the period of observation and was slowly becoming an ecosystem where a diversity of animals, plants and bacteria exist. The observed plants and animals existing in the sedimentation pond include bloodworms, sweep, abalone, sea snails, sea slugs, brown benthic algae, *Ulva sp.*, macroalgae, green benthic algae, copepods, isopods plus an array of microscopic organisms. It can be assumed that these plants and animals were contributing to the degradation and utilisation of the particulates and nutrients that were delivered to the pond from the farm.

Total farm dissolved phosphate concentrations increased above ambient concentrations from September 2003 onwards and this increase is likely to be attributable directly to the rate of supply of the artificial diet which related well to the total farm dissolved phosphate effluent concentrations. Further supporting this idea is the formulated feed stability trial which found a 26% leaching of P within the first 15 minutes (Chapter 2.1). The source of this P may be due to P supplements which are commonly added into abalone diets. It is known that manufacturers of artificial diet will often add excess P to avoid any growth problems as the availability of inorganic P is

usually not known and difficult to determine (Sales et al., 2003). The artificial diet used by AFA contained 0.68% P part of which was supplemented with a soluble P source (supplement constitutes approx 0.2% of the 0.68% or 29% of the total P content) (Scanlon, personal communication). This is consistent with research by Coote et al. (1996) who found that supplementing P to 0.7% in diets increased growth rates by as much as 7.9%. Given abalone are slow and messy feeders, coupled with their nocturnal feeding activity, it would stand to reason that some of the soluble source of P may leach from the diet before the abalone are able to ingest it (Chapter 2.1). This may well be occurring year round as even during winter (i.e. time of the year when time between feeding and the commencement of feeding by the abalone is shortest) there is often a 2-3 hour window between feeding and consumption of the diet. Hence, some of the phosphate supplement is likely to have leached into the water column leaving mainly the base diet P (fishmeal and soya component). This claim may be further supported by the relatively low rates of leaching of P exhibited by the reference diet (i.e. no P supplementation) in Coote et al (1996) which contained soya meal and fishmeal only (personal communication). Sales (2003) using similar P supplements found that between 29.9 and 33.4% of P may be leached within the first hour. Further evidence from the 24 hour sampling trial was the peak in phosphate concentration at the farm outflow at 8am when feeding occurred at 3pm the previous day (Chapter 2.3). This coincides with the residence time of water on the farm.

Unlike the ammonium, the tank phosphate concentrations were not well correlated with the total farm phosphate concentrations. Perhaps this indicates that the feed in the tanks were not influencing the total farm concentrations of dissolved

phosphate but that would be both contradictory to the evidence above and illogical given that the biggest source of P is the feed input. The proposed explanation for this discrepancy is the time of sampling. In general sampling occurred between the hours of 10:00am and midday and the P leaching quickly from the diet put in the evening before would have been largely flushed from the tank systems by the morning (hence the lack of correlation).

The production of phosphate in the sedimentation pond is likely to be due to the high levels of particulate P that were found in the tank waste. The particulate material from the tanks was flushed twice a week and settles in the sedimentation pond (Chapter 2.2). The average P concentration of the tank waste was 4694ppm, while the average sedimentation pond sediment concentration was 2663ppm representing a potential 2031ppm loss of P from the particulate phase (Chapter 2.2). This loss may be due to remineralisation and export into the water column and then export to the marine environment.

Unlike N and P, silicate concentrations within the farm system showed no relationship with feed input. Alternatively the peaks in total farm silicate production may be primarily attributed to rainfall. Literature suggests that rainfall has practically no silicate (Asano et al., 2003) however the correlation between sedimentation pond production and rainfall may be attributable to the terrestrial clay and particulates which were washed into the sedimentation pond during heavy rains. While there was no rain on the day of samplings (except one period where noted as an outlier) and salinity of all but one sample was 35ppt, the increased terrestrial particulate loading may have caused remineralisation of silicate into the water column.

Other sources of silicate within the farming system include the cement tanks and also the formulated feed. The artificial diet contained low levels of silica (130ppm) and had the capacity to raise the farm effluent concentrations by up to 0.0165μm/L ambient seawater (assume total dissolution and 30kg feed/day and 8.6 ML daily water flow see Appendix). The empty tank trial showed between 34-42g (240-300μmol/L) of silicate leaching from a newly constructed tank over a 3 day period. The tanks had the capacity to raise the total farm effluent by 0.104 - 0.131umol/L above ambient seawater, or about 8 times more than the possible input from feed. While this gives little indication as to the long term leaching it does point to the cement as a source of some silicate. In addition to this as the abalone graze the tank walls and as particulates are transported into the tanks (through wind, wave action at farm inflow) some silicate may be remineralised into the water column and could account for some of the production within the tanks. Of some importance, however, is that there were only 2 cases when the concentrations of silicate were greater than the highest ambient concentration at the farm inflow indicating the ecological significance of these results may not be in the concentrations but rather the timing of the elevated concentrations (i.e. high concentrations may occur at outfall when low concentrations may occur at the farm inflow).

There was only a single case of net particulate load produced by the farm. This occurred during the construction of the farm where freshwater runoff entered the main drains carrying terrestrial silt and clay. This freshwater caused increased nutrient concentrations in the sedimentation pond and demonstrated that the sedimentation pond could be inefficient for the removal of particulates in freshwater, probably due to a halocline forming within the sedimentation pond (farm discharge is drawn from the

surface layer of sedimentation pond). In terms of organics the farm outflow POM was never statistically greater than at the farm inflow. Despite this there were many cases where mean POM at the outflow was greater than at the inflow. If organic loading to the environment is occurring it is likely to be driven by many dynamic processes both internal and external to the sedimentation pond that are difficult to characterise. The internal factors are likely to be the biological processes/blooms occurring and the external are likely to be factors such as wind causing resuspension of particulates and feeding practices on farm.

While the consumption of the measured nutrients within various compartments is an important process within the farm, it should be noted that those nutrients may not have been eliminated from the system but rather converted into another form. This form may be an unmeasured form, or more likely be bound biologically. Some of the unmeasured sinks of these nutrients may be through denitrification (likely to be of relatively low significance) and effluent discharge of phytoplankton (i.e. while there is no net increase in particulates there is a compositional change in the particulates exiting the farm). Of these possibilities, the net export of phytoplankton represents the most likely scenario (based on previous evidence and visual differences of farm inflow and farm outflow filter paper samples).

3.4.1 Total farm N budget

The total farm nitrogen budget gives an approximation of the pools of nitrogen for AFA with an abalone biomass of approximately 18-20 tonnes. The approximate abalone nitrogen recovery ranges between 460 kg based on the figures from Neori (2000). The

figures from Neori (2000) were used to calculate the nitrogen retention within the abalone as that study fed abalone enriched *Ulva sp.*, a macroalgal diet which has been shown to have similar growth rates to Adam and Amos artificial diet (diet used at AFA) (Boarder and Shpigel, 2001).

In the farm budget the net annual export of nitrogen in the particulate form was 0 kg. From ten samplings there was only one case of a statistically significantly net particulate export during both periods when the farm was being cleaned and when the farm was not being cleaned (Ho et al., 2003). In reality there may well be occasions where despite no statistically significant output, either finer particulates may be exiting the farm or there is an increase in the nitrogen composition of the particulates exiting relative to the particulates entering the farm (i.e. the method of Franson (1989) which uses weight difference for detection may not be sensitive enough). The latter of these possibilities is highly plausible given the nitrogen addition (through feed input) into the farm system. Thus particulates could potentially represent a substantial export of N given the volumes of water usage on the farm (annual usage = approximately 3153 megalitres) and may warrant further investigation.

The dissolved nutrient fraction of the budget, which accounts for 31%, can be attributed to the excretion by abalone and the breakdown of particulate matter within the compartments of the farm system. The relative proportions attributable to each source are unknown; however the amount of ammonium directly excreted across the gills of the abalone is likely to be reasonably small relative to the amount of ammonium leaching from the faeces and uneaten feed (Barkai and Griffiths, 1988). In addition, the dissolved fraction does not account for any lag phases between addition of particulates into the

system and remineralisation of those nutrients into the dissolved fraction (i.e. is a snapshot taken over a 2 week period)

Cleaning within the farm has been shown to increase the level of effluent nutrients (Ho et al., 2003). Despite this the data used to compile the dissolved NH_3 and NO_x (nitrite+nitrate) fraction does not incorporate data for cleaning. Cleaning did not constitute the major time fraction of the farm operations (under 7% of the week is spent cleaning) and the exact dynamics of a cleaning cycle are difficult to fully characterise (i.e. concentrations are highly variable in time and were not characterised). Thus the figures for effluent discharge given are likely to be underestimated. In addition to this, other dissolved sources such as proteins and amino acids have not been measured hence further leading to underestimation of the total dissolved fraction.

The particulate fraction held within the system (approximately 45% of Nitrogen) is derived from the particulate waste experiment and is predominantly composed of faeces and uneaten feed. The estimated particulate load is also likely to be underestimated due to the fraction smaller than 250μm not being retained (screen size) or quantified.

Overall the budget represents a 'snap shot' representation of AFA's nitrogen inputs and outputs at one point in time (extrapolated to an annual budget for a farm with an 18-20 tonne abalone biomass). That the budget does not balance is probably due to the undescribed relationship between the dissolved fraction and both the particulate pool and abalone metabolic waste. If we assume 100% of dissolved nitrogen is derived from the particulate pool remineralisation, then a 210kg-N deficit (or ~17%) exists. In reality only a portion ~50% by concentration – particulate waste trial) of the nitrogen in the sedimentation pond is likely to be remineralised as dissolved nutrients. Specifically

caution must be applied to the dissolved export and particulate fraction for reasons stated above. Also it should be noted that the components displayed will vary through time depending on farm practices and environmental conditions and the results while broadly applicable to most farms are still likely to differ between farms.

3.5 Conclusions

The results of this study indicate that different compartments within the farm have different effects on the total environmental performance of AFA. In general the tanks and sedimentation pond produced ammonium, nitrate, nitrite and silicate while the drains consumed these nutrients. These findings indicate the importance of farm design on nutrients exported as potential causative agents of environmental impacts (i.e. dissolved and particulate nutrient release) may be eliminated through careful system design. In particular the production of ammonium, nitrate, nitrite and silicate within the sedimentation pond system highlights the need for future work to avoid any potential environmental impacts which may be caused through nutrient release. This work also raises the question of the benefits of sedimentation pond given they may produce and export dissolved nutrients. It is our opinion however, that solids removal devices (i.e. sedimentation ponds, solid separators or other devices) are likely to be important to prevent the localised 'blanketing' of marine life surrounding the discharge environment (a 20 tonne farm is likely to produce approximately 6000-8000kg of particulate waste with a N content of 213-260kg N, 1044-1392 kg Carbon, 28-37kg P). Such deposits of particulate waste may cause problems such as eutrophication (Bergheim and Brinker, 2003) and increased BOD (Amirtharajah and O'Melia, 1990).

Nitrogen and phosphate within the abalone farming system show strong correlations with feed input. This suggests that the management of these nutrients is a direct result of the formulation of the feed and the husbandry used to deliver the feed. As abalone nutrition advances we an expect to see lower wastage of the feed. In particular a source of concern is the wastage of P as shown in the formulated feed stability trial (Chapter 2.1) and also in the results of this chapter. Clearly any wastage of nutrients is a source of potential environmental damage (concentration and load dependent) and where possible steps should be taken to eliminate any unnecessary nutrient pollution.

CHAPTER 4: Environmental Impact Study of effluent receiving environment

4.1 Plume study

4.1.1 Aim:

To determine the dilution rates and dispersion of the abalone farm effluent once discharged into the marine environment

4.1.2 Introduction

A study was conducted to determine the likely areas of impact under the most representative of sea conditions. This was completed to determine the dilution of the abalone farm effluent and what concentrations were remaining around the farm outfall, along the shoreline or out towards the sea. This then gives perspective as to the ideal sampling design for a long term environmental impact assessment monitoring program. In addition the experiment gives insight into the likely 'impact zone' which the effluent will extend to. Therefore if we can establish the degree of dilution we can predict at what distance there is likely to be no measurable increase of the effluent on receiving waters. Relatively calm weather was chosen as the most appropriate time to conduct the study for both logistical and representative reasons which are demonstrated below.

Tracer dyes have many applications however a common application is to assess the movement of water within or through an environment (Gaikowski et al., 2004; Nameche and Vasel, 1998). The fundamental principle employed by many tracer dyes is the phenomenon of fluorescence which can quantitatively be measured using a

fluorometer. The dye may be added at a known location at a known concentration, measured and then re-measured at other locations making it possible to calculate dilution or dispersion of the source water during the process of mixing with the receiving waters.

While there are many dyes available on the market, in recent times Rhodamine WT ($C_{29}H_{29}N_2O_5.Cl.2Na$) has been the choice of tracer dyes for wastewater studies in the ocean primarily due to a few key properties which other dyes lack. Rhodamine WT disperses readily and is more stable when exposed to particulate matter and UV light (Guilbault, 1990) and easily detected with a fluorometer to levels as low as 0.01mg m^3 (Kasnavia et al., 1999). Of the greatest concern with other available dyes such as fluorescein is that they tend to adhere to particulates (Wilson et al., 1986) and hence presents a potential loss of tracer dye. This can present problems particularly in quantitative studies.

Studies employing tracer dyes can be both quantitative and qualitative in design; however, each study should be conducted separately as different concentrations of dye are required for each type of study (Barter, 2002). For quantitative studies dye should be delivered at a constant concentration which is determined by the stability of the receiving waters, however as a general rule approximately 100ppb should be the maximum concentration within the receiving water as below 100ppb Rhodamine WT fluoresces linearly (Turner Designs, 2005). On the other hand for qualitative studies a one off 'slug' injection is applied and initial concentrations are planned to be above 100ppb (Barter, 2002). This ensures that the dye remains clearly visible within the water and hence the initial movements of the water and its dye tracer are apparent. At concentrations >100

ppb aerial photography is commonly used as a means to temporally and spatially track the movement of the dye in the receiving waters.

4.1.3 Materials and methods

4.1.3.1 Local conditions

Five years of data from the Bureau of Meteorology (BOM) was obtained for the Bicheno weather station where twice daily observations of the swell height and direction are recorded. This data was sorted and the incidence and occurrence of each condition was calculated for the three year period to December 2003. A description of each condition is given in Table 4.1:

Table 4.1: Description of the different categories for sea conditions. Source = Bureau of Meteorology

Description	Height (metres)	Effect
Calm (glassy)	0	No waves breaking on beach
Calm (rippled)	0 - 0.1	No waves breaking on beach
Smooth (wavelets)	0.1 - 0.5	Slight waves breaking on beach
Slight	0.5 - 1.25	Waves rock buoys and small craft
Moderate	1.25 - 2.5	Sea becoming furrowed
Rough	2.5 - 4	Sea deeply furrowed

4.1.3.2 Plume mapping

On the day of the study (conducted during February 2004), farm water flow rates were calculated by measurement of the main drain. In the main drain (all seawater used on the farm flows into this drain, into the sedimentation pond and out of the farm outflow) the width and water height of a 10m section were measured giving an indication of the volume of water held within this 10m section. A float then placed at the start of the 10m section and the time taken for the float to travel the 10m distance was recorded. Based on these drain measurements the farm water flow rate and the amount of Rhodamine WT required to achieve an initial concentration of 100 ppb was calculated. The appropriate amount of Rhodamine Wt was then added directly to the effluent (Fig.

4.1). Approximately parallel to the shore a100m transect line was laid out extending 50m either side of the end-of-pipe. The end-of-pipe faces approximately ESE and the contour of the coastline can be seen in Figure 4.1.

Before the dye was added to the effluent water, samples for background fluorescence (caused by microalgae, organics and minerals (Wilson et al., 1986)) were taken in 120ml polystyrene jars. These samples were taken at 4 points along the transect line in triplicate and the average of these values subtracted from the other values recorded. A peristaltic pump powered by a Robin 500 watt generator delivered the Rhodamine WT directly to the effluent water. This was achieved at a point 10 metres after the sedimentation pond outflow (i.e. at a break in the pipe between the sedimentation pond outflow and the end-of-pipe) (see Fig. 4.1). The Rhodamine WT was injected into the water for approximately 60 minutes before sampling in the receiving waters began. Water samples at the beginning and end of the study were taken in triplicate directly from the end-of-pipe as a reference. The resulting value was averaged and is represented as the end-of-pipe value in the text. Samples were then taken (in triplicate) at 10 metre intervals along the 100m transect. Further samples were also taken 30m out to sea from the end-of-pipe at three points, 30m on a south easterly bearing, 30m due east and 30m to the south. Samples were taken mid water column (~ 3m depth) using a Niskin water sampler. All samples were stored in the dark and transported directly back to the laboratory for analysis on a Turner Designs 10-AU fluorometer.

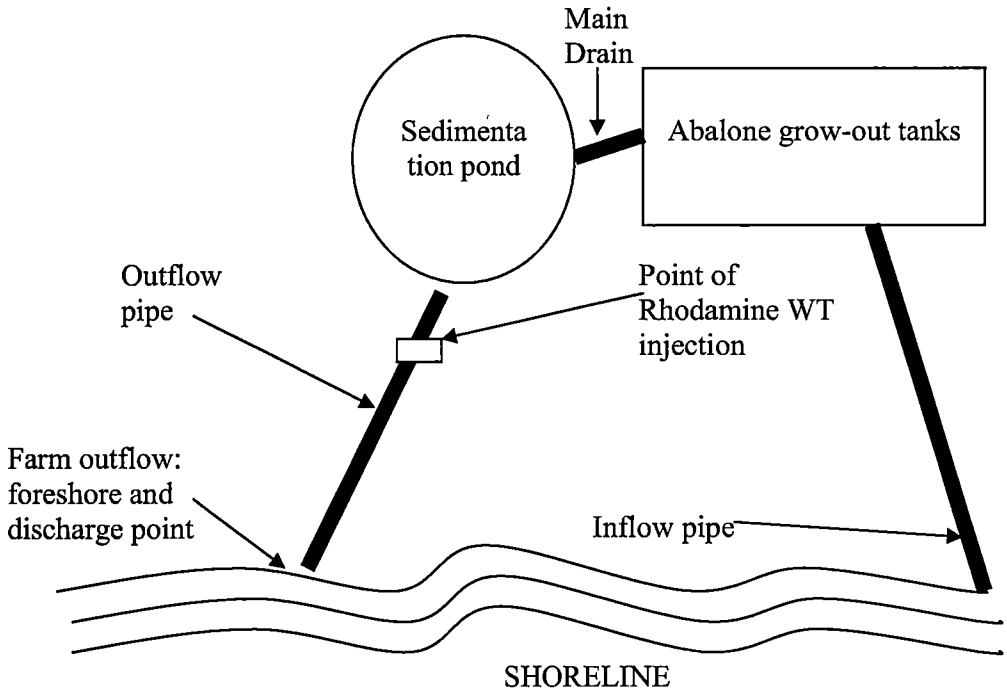

Figure 4.1: Schematic of farm illustrating water flow through the farm and the point of Rhodamine WT injection into the effluent

Temperatures were recorded at the farm inflow and farm outflow waters using a YSI 6600 sonde data logger as possible temperature differences between the receiving waters and the effluent water may result in a reduced rate of vertical mixing.

4.1.4 Results

4.1.4.1 Local Conditions

The data in Figure 4.2 shows the conditions as measured by the Bureau of Meteorology over a 5 year period to December 2003. These data shows that smooth

wavelets are the most common sea condition occurring 38% of the time over this period. Slight and Moderate conditions account for approximately one half of the conditions over this period while Calm (rippled) conditions occurred 12% of the time. Calm (glassy) and Rough conditions occurred under 3% of the time. On the day of the plume study 'Smooth (wavelets)" were observed as recorded by the BOM. The swell conditions on the day of sampling was 1m southerly (offshore observation), but in the coastal region were the plume study took place the swell was much smaller (about 30cm in height).

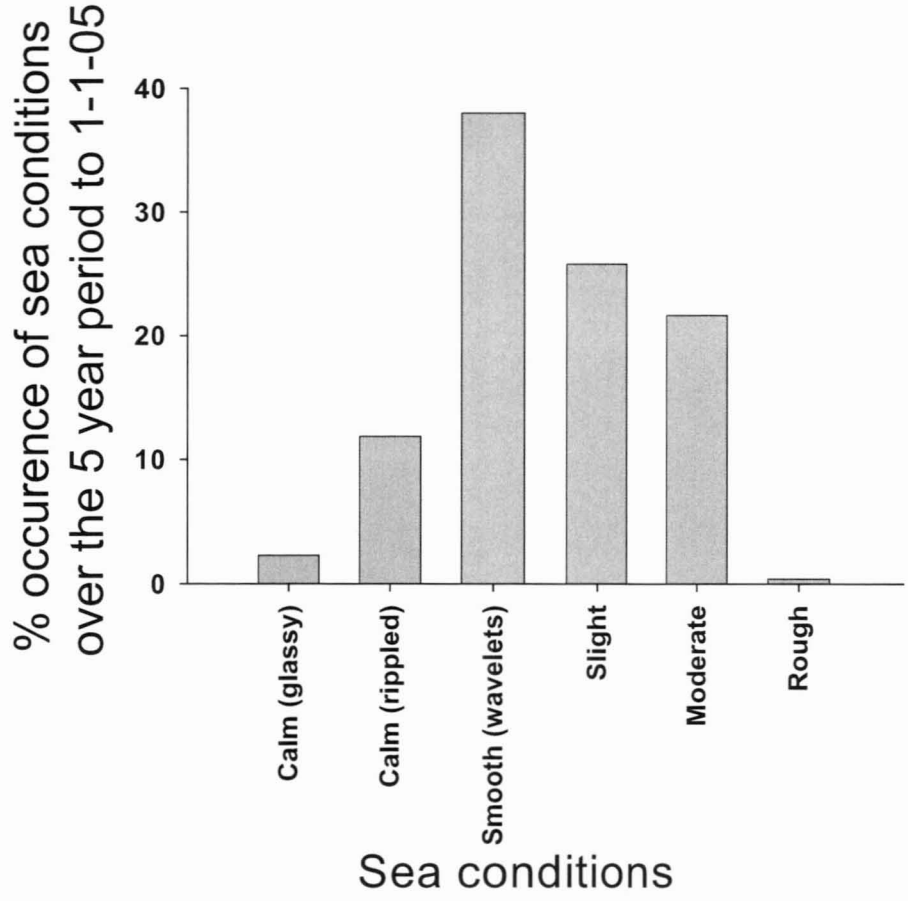

Figure 4.2: Occurrence of sea conditions observed in Bicheno over the 5 year period to 1st of Jan 2004. Data source = BOM.

On the day of the trial the wind conditions were calm (9am BOM observation) to SE (3pm BOM observation) winds which were between 5-15 knots. The percentage occurrence of the spectrum of wind directions from the BOM indicates that the predominant conditions are Calm and NNE winds which both occur 15% of the time (Fig.4.3).

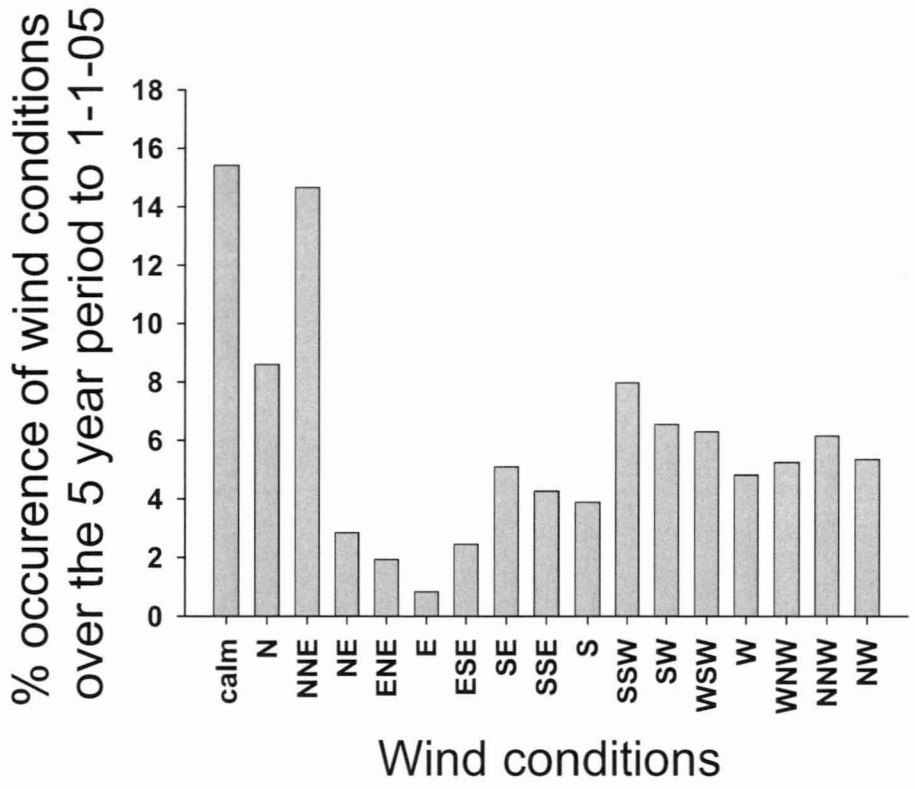

Figure 4.3: Occurrence of wind conditions observed in Bicheno, Tasmania over the 5 year period to 1st of Jan 2004. Data source = BOM

Temperatures at the farm inflow relative to the end-of-pipe were within 0.10 of a degree at the time of sampling and this small difference was statistically significant

($P<0.05$). The average temperature at the farm inflow was 16.10 ± 0.017SE (N= 53), and at the end-of-pipe it was 16.17 ± 0.001SE (N=53).

4.1.4.2 Plume mapping

The dye concentration results indicate that the plume was predominantly moving in a south/south westerly direction once leaving the end-of-pipe (Fig. 4.4). Background fluorescence measured 7.0 ± 1.2 ppb as measured at 4 different points along the transect line. Concentrations (after blank subtraction) decreased from 64.3ppb at the end-of-pipe to 32.5ppb 10m south of the discharge; while 10m to the north substantially lower values of 10.8ppb were recorded (Fig. 4.4). This represents 50.6% and 16.7% of the end-of-pipe Rhodamine WT concentration for the south and north respectively. Further past the northern 10m point the Rhodamine WT concentration did not decrease (statistically significantly) with distance and no gradient was visible out to 50m. Twenty meters south of the end-of-pipe the Rhodamine WT concentration decreased to 21.9ppb which represented 34% of the end-of-pipe concentration. By 30m south the concentration of Rhodamine WT decreased to levels 9.4ppb or 14.6% of its original concentration. Past 30 m south the concentration of Rhodamine WT was not significantly different than the 40m and 50m southern points.

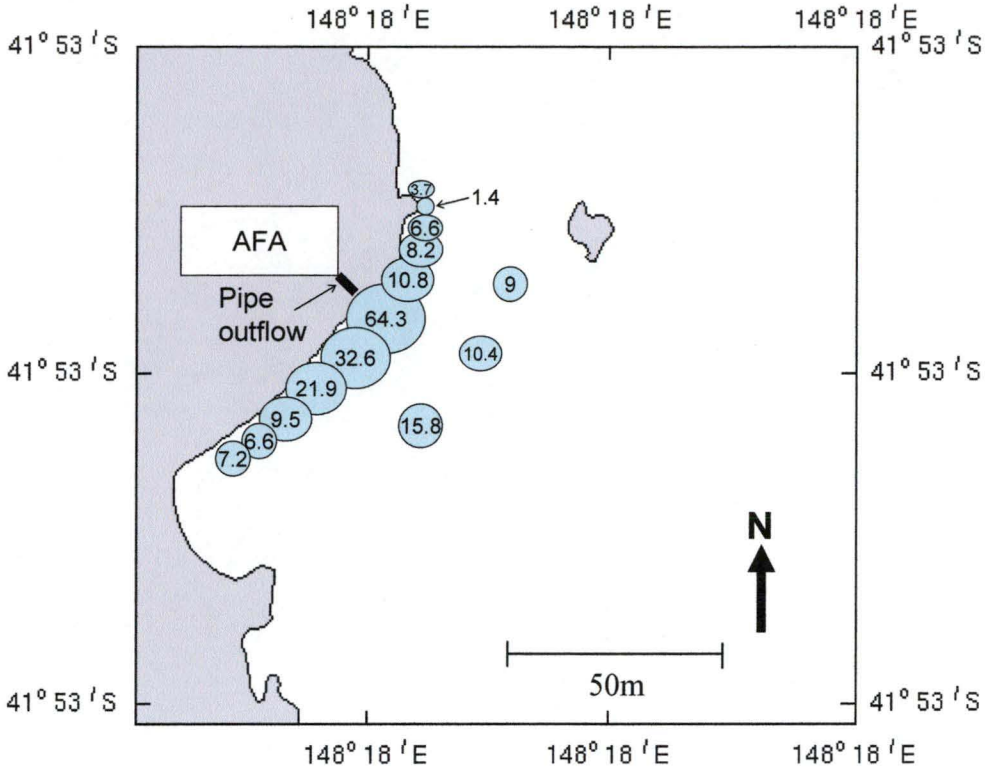

Figure 4.4: Bubble plot of Rhodamine WT concentration (mean values in ppb are listed within the bubble, *n*=3) versus positions along a 100m transect and 30m out to sea.

Figure 4.4 shows a decreased concentration of Rhodamine WT for the three points 30m seaward from the end-of-pipe (eastern, south eastern and southern). Both the eastern and the south-eastern 30m samples showed Rhodamine WT concentrations of 9 and 10.4ppb respectively compared with the end-of-pipe concentration of 64.3ppb. This equates to 14 and 16.2% of the original end-of-pipe concentration. The southern 30m sampling point showed a slightly greater mean concentration of Rhodamine WT with 15.8ppb which represents 24.6% of the end-of-pipe concentration. These seaward samples were taken approximately 20 minutes after the initial concentration of Rhodamine WT at the end-of-pipe was taken.

4.1.5 Discussion

The results of this trial indicate that under the most commonly observed sea conditions (smooth = 38% of local BOM observations) the intertidal area within 50m from the point source can have between 94.1 to 88.8% lower concentrations of effluent than at the outfall. Under the trial conditions the effluent was more concentrated in the southern area of the intertidal region with elevated concentrations of Rhodamine WT (relative to the northern area) and decreasing concentrations detected between the end-of-pipe and 30m south. More than 10 m north of the outfall there appeared to be little evidence of changes in concentrations of Rhodamine WT as they were statistically similar for all sampling points to the north. This suggests that the effluent is flowing relatively cohesively in the north when compared to the southern areas. Despite this average Rhodamine WT concentrations decreased with distance from the outfall; however, perhaps only at rates not statistically detectable due to the relatively small differences between concentrations at difference distances.

The effluent was reduced to approximately 13-24% of the end-of-pipe concentration by 30m seaward. In particular the east and south easterly seaward samplings were between 13 and 16% of the end-of-pipe concentration; however, the southerly reading was much greater in concentration at 24%. This indicates a greater tendency for the effluent to move in a southerly direction out to sea, a finding consistent with the results from the intertidal sampling results (i.e. intertidal results suggests a southerly moving plume). The trial does not also account for vertical mixing of the effluent within the water column. Whilst this is an important component of the mixing,

the risk of error in sampling has been minimised by sampling mid water column. It would also appear that more of the plume may be flowing out to sea (in a southerly direction) than parallel to the shore in the intertidal region (as the 30m southern seaward point was almost 100% greater than the intertidal 30m for both southern and northern sampling points). This is a surprising result given the dilution factors associated with the plume moving out to sea, relative to its movement within the intertidal region. The greater seaward Rhodamine WT concentrations may be explained by tidal influence as the tide was slack (high) when the experiment began, but over the duration of the experiment (and by the time boat sampling occurred 30m to sea) the tide had shifted to an outgoing tide. Another possible explanation for the greater seaward rhodamine concentrations is the assumption that the intertidal region and the points 30m to sea are similar in background fluorescence (i.e. background fluorescence measurements taken only from intertidal and applied across all samples). In reality if a different fluorescence matrix between the intertidal and subtidal areas existed, this would likely have a considerable effect given the magnitude of the subtidal sample concentrations relative to the background fluorescence (i.e. background fluorescence constitutes approximately 20% of the subtidal samples).

Based on previous BOM records, the sea conditions under which the study was conducted was by far the most dominant sea condition occurring 38% of the time. While this equates to approximately 138 days per year it cannot be safely assumed that the plume will disperse similarly on all these days. Many other factors such as tide height and time, wind direction, swell and thermal differences between the effluent and receiving waters are likely to influence dispersal. Under the conditions of 'smooth (wavelets)' an

important factor in determining dispersion is the thermal differences of the farm outflow and farm inflow water. While on this occasion there was only a slight temperature difference between the effluent and the farm inflow water, a temperature difference of up to 2 degrees has been noted on various occasions presumably due to ambient air temperature, solar irradiance (causing warming of the cement tanks and subsequent energy transfer) and long residence time of farm water. The temperature determined density difference is likely to result in a thermocline which may temporarily inhibit the mixing of the effluent water with the ambient water around the farm outfall. During summer it is likely to cause the effluent to reside on top of the receiving water and under these circumstances, wind direction may have a greater role in determining the direction of the plume and dispersion of the effluent, when compared with winter conditions. Overall, based on sea conditions and the wind conditions the results of this trial are likely to be representative of the dominant conditions which affect the coastline directly in front of Abalone Farms Australia.

For approximately 14.2% or 51 days per year the conditions were calmer indicating that the results of this trial are perhaps not the absolute worst case scenario with respect to effluent dispersion. Approximately forty eight percent (47.8%) or 171 days of the year the surface waves are larger and the effluent plume is likely to be dispersed at a greater rate than in this trial. With respect to wind conditions, the trial was conducted under winds both calm and SE in direction. Wind has the ability to affect surface plume spread (Elliott and Wallace, 1989) depending upon the direction of wind relative to the direction of current and hence plume movement. Given that the plume was moving in a S/SE direction, it is likely that a SE wind may cause movement of the plume

to be slightly slowed or restricted. Under other wind conditions such as a northerly wind, the dispersion of the plume may be enhanced in a southerly direction.

The findings of this trial indicate that the of the end-of-pipe effluent concentration of any dissolved contaminant is likely to be reduced to less than 10% and 25% beyond the boundaries of 50m in the intertidal and 30m out to sea, respectively. Hence when talking about environmental impacts, if we assume a constant reduction in effluent concentrations with distance, we can safely say that past 50m there is likely to be a minimal effect of the abalone farm effluent on the marine environment (i.e. detectable at less than 10%) for the majority of the weather conditions. However despite this it needs to be stated that the plume study does not represent the worst case scenario for all sea conditions and hence at other times the effluent may be detectable at distances greater than 50m.

4.2 Environmental Impact Monitoring Program

4.2.1 Aim:

To characterise the biological impact of AFA's effluent waters on the local marine environment adjacent to the end-of-pipe outflow.

4.2.2 Introduction

As the Australian abalone aquaculture industry expands, there is a need for the industry and government to work together in defining what environmental requirements should be in place to ensure a sustainable industry. To date the environmental effects of land based abalone farming are still unexplored with no published Environmental Impact Studies (EIS) available. Without such studies the biological significance of farm effects of the on the local marine environment remains unknown.

The land based nature of many abalone farms provides an easy means of studying benthic impacts. Typically, abalone culture occurs in facilities where water is pumped from the ocean to the culture tanks (which vary in description from shallow plastic trays, to PVC pipes to deep water cement tanks). In some cases the water is then treated by a wetlands area which may be a sedimentation pond (in other instances maybe an intertidal sandflat area or Solid Separation Device (SSD)) or is returned to the ocean without solids removal occurring. The water is usually returned to the ocean by a pipe (point source discharge) where it is diluted and mixed into the receiving waters and the effluent assimilated into the marine environment. Hence, a study of the surrounding intertidal region and the nearshore subtidal region for community shifts relative to a control or a

pre-discharge study will provide an indication of how the farm effluent is interacting with the marine ecosystem.

Given the extremely high diversity and endemism of southern Australia's temperate marine communities (Underwood et al., 2000; Womersley, 1981) detecting impacts is often a difficult task (Crowe et al., 2000; Kingsford, 1998; Underwood, 1991; Underwood, 1992; Underwood and Chapman, 2003). Traditionally studies have employed control versus impact site designs, with before and after comparisons (BACI) if possible. However, due to the highly variable nature of benthic marine communities (both spatial and temporal), natural variation between control and impact sites can be substantial. Thus the need for multiple control sites has been argued (Stewart-Oaten et al., 1986; Underwood, 1991; Underwood, 1992). Ideally control sites would be identical (biologically and physically), as well as subject to the same environmental conditions. It has long been recognised that sea conditions (i.e. exposure, shore orientation) are important for the selection of control sites as water motion has long been recognised as a major determinant of intertidal communities (Lobban and Harrison, 1994). Clearly no two sites can be identical, however if the impact signal is strong and control sites are relatively similar, detection of an impact is possible. Another important factor associated with the impact is the composition of the effluent being discharged. Some possible effects of aquaculture effluent include the oxygen 'starvation' of other aquatic organisms (increased Biochemical Oxygen Demand (BOD) (Islam et al., 2004; Michael Jr, 2003; Teichert-Coddington et al., 1999), eutrophication (through increased nitrogen and phosphate loading) (Crawford, 2003a; Porrello et al., 2003), reduced assimilation capacity (possible temperature and/or salinity differences between discharge and

receiving environment causing a thermocline), and the possible 'blanketing' of marine life through increased particulate loading (Loch et al., 1996). Any one or combination of the above has the potential to impact upon the marine life and cause a shift in the functionality and structure of the environment in an 'unnatural' way.

The changes observed within an impacted environment are likely to be a function of the resources that are input into the environment. If, for example, the ammonium produced by the abalone farm results in a significant increase in concentrations around the vicinity of the discharge point, then the most efficient users of this resource (when coupled with the local environmental conditions) are likely to proliferate relative to the control sites. Typically under conditions where there is strong environmental change R strategists are likely to flourish (Barnes et al., 1996) due to their capacity to reproduce quickly and produce large amounts of offspring (Pianka, 1970). As the response of some species to enrichments of various nutrients is already known, characterization of the effluent will in itself give some indication as to what species present at the impact site are likely to proliferate. The species may be broken into a number of functional groups and for the purposes of this study the three main groups are:

1. nutrient scavenging macrophytes
2. particulate filter feeders
3. grazers

These groups were decided based upon the dominant species present at the outfall and control sites and the functional ecological niche these species fill.

At Bicheno the recorded nutrient scavenging macrophyte group is made up of primarily *Ulva* and *Porphyra* sp. both of which have the capacity to capitalise on high levels of nutrients (Chopin et al., 1999; Malta et al., 2002; Pedersen et al., 2004). The dominant particulate feeders at the impact site are the intertidal mussel *Xenostrubus pulex*, and barnacles such as *Caterornerus polymerus* and *Chamaesipho tasmanica.* Finally the grazers are mainly from the subclass Prosobranchia and composed of limpet species and periwinkles species, as well as a few other species from other taxonomic groups such as Chitons and Asteriods. These species are likely to be primary consumers of microalgal and/or bacterial populations at the impact and control sites (Edgar, 2000).

Another means of examining the effect of anthropogenic disturbance such as an abalone farm is through diversity indices. Communities which have high levels of anthropogenic disturbance are likely to experience decreased levels of diversity (Sax and Gaines, 2003) , which can in turn affect ecological processes (Duarte, 2000). This is also true for a number of aquatic studies which have reported decreased diversity with eutrophication (Cederwall and Elmgren, 1990; Sundback and Snoeijs, 1991; Worm et al., 1999). A number of diversity indexes are available for use each with different applications and meanings. One of the more commonly used indexes is Margalef's diversity index which gives an indication of the number of species present for a given number of individuals counted (Clarke and Warwick, 2001) and hence offers a good description of species richness for monitoring programs through time (i.e. temporally variable numbers of species and individuals).

Abalone Farms Australia (AFA) operates from Bicheno on the east coast of Tasmania, Australia. The farm management are conscious of their environmental

performance and have constructed an extensive wetlands area consisting of three ¼ of an acre lined sedimentation ponds connected into a system that sequentially receives and treats the farm's effluent prior to discharge into the local marine environment. This study looks at the environmental impacts of AFA's land based farming operation at the discharge point into the intertidal region of a rocky foreshore on the East coast of Tasmania, Australia.

4.2.3 Materials and Methods

4.2.3.1 Quadrat monitoring information

AFA's effluent discharges at the low water mark along an exposed coastline on the East Coast of Tasmania, Australia (41°53.367S, 148°18.374E). Control sites were established approximately one kilometre in a northerly (41° 52.768S, 148°18.527E) and southerly (41°53.814, 148°18.524E) direction (Fig. 4.5). Distances of 1km were chosen as this was deemed to be far enough away from the abalone farm so as to not be affected by the effluent, but close enough such that similar oceanic conditions to the farm discharge site are exhibited. The discharge and control sites are characterised by rocky granite boulders, east/south easterly exposure and large bull kelp beds (*Durvellia potatorum*) at the low water mark. Each of the control sites were free from disturbance (i.e. located at least 500m from any industry or potential pollution source) from other industry such as seafood processing and aquaculture which occur at some locations along the Bicheno coastline.

At each site, the intertidal region was divided into 2 littoral zones. The lower littoral zone extended between the low tide mark and upper limits of the mid intertidal region and was characterised by predominant foliose macroalgal populations, grazing molluscs and barnacles. The upper littoral zone extended between the upper limits of the mid intertidal mark and the high tide mark and was characterised by the presence of littorinid prosobranchs and lottiidae barnacles.

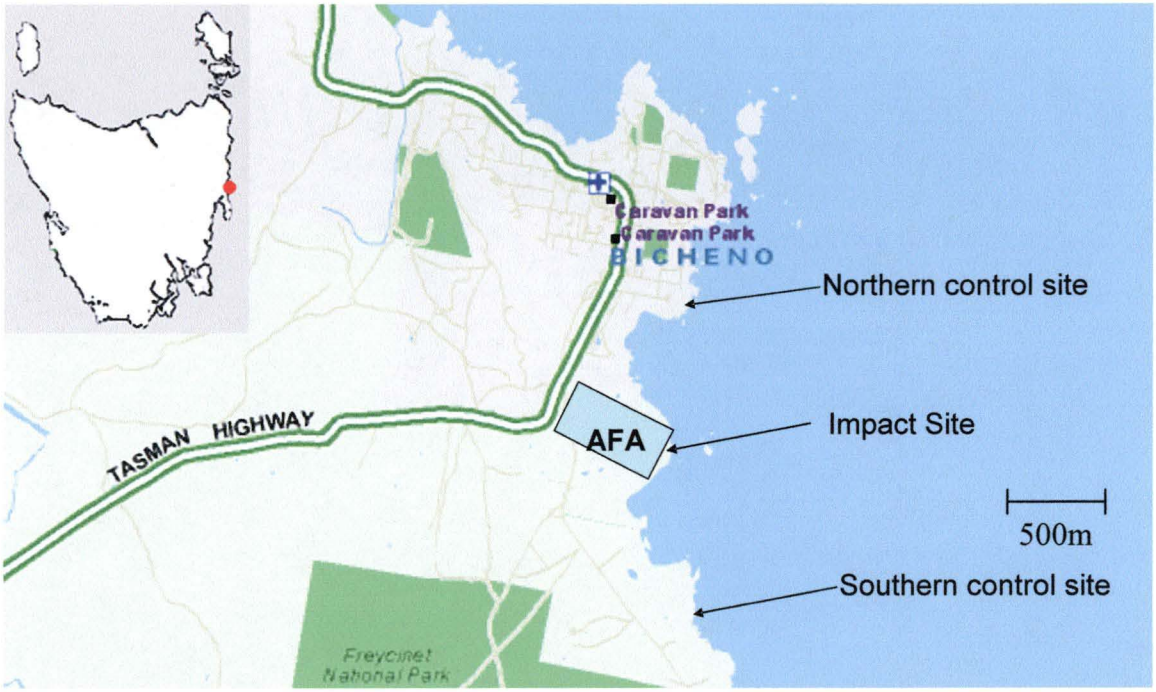

Figure 4.5: Map showing the location of AFA and the southern and northern control sites along the Bicheno coastline.

A series of 10 quadrats (1m x 1m) were randomly placed within each of the littoral zones (i.e. upper and lower). If a quadrat landed on a rock-pool, the quadrat was re-thrown. The precise position of the quadrat was marked out using an epoxy glue to

mark the 2 corners of the quadrat on the landward side. For each quadrat total invertebrate and macroalgal populations were recorded (details below). Samples of each species observed were taken, identified and stored in addition the entire quadrat was photographed. Sampling of all sites was conducted 3-4 times per year; however, due to problems associated with the epoxy glue used for the southern control site, before photographic data are not available. The quadrat marks were re-established at the second sampling time at this control site.

4.2.3.2 Counting

Total counts of all organisms were made for every species within the quadrats. Where possible individuals were counted. *Porphyra* and *Ulva* were measured by both individual counts and % cover estimation. For *Chamaesipho tasmanica* individual counts were often difficult due to the dense concentrations of this species. Consequently counts of 10 squares (10cm x 10cm) of the 100 squares within a quadrat were made, averaged and the resulting figure multiplied by 10 to give a density per m^2.

4.2.3.3 Transects

A transect was monitored to determine if significant changes occurred in the subtidal macroalgal assemblages and if these changes may be linked to the operation of the abalone farm. A 50m seaward video transect was completed at the impact and control sites, both before the farm discharge had been commissioned (May 2002) and at the cessation of the quadrat monitoring program (June 2004, i.e. after 2 years of farm

operation). Transect line positions were orientated using compass bearings from fixed points on the foreshore. Video footage was taken along the entire 50m transect ensuring that at least 2m either side of the transect line was visible. At 10m intervals video footage of the area surrounding both sides of the transect line was recorded covering an approximate $9m^2$ area. For all sites qualitative data (i.e. the relative cover of canopy forming macroalgae) was transcribed from the video footage and recorded for each of the $9m^2$ areas.

4.2.3.4 Local environment conditions

The marine environmental conditions at Bicheno are important for an understanding of the community structure within the intertidal rocky shores. Bicheno is located on the east coast of Tasmania, Australia (latitude: 41.8756 S, longitude: 148.3022 E). It is a temperate exposed coastline where water temperatures commonly range between 12-21°C. The predominant swell is from a north/north easterly direction and seas are usually between 0.1 and 0.5m in swell height. Bicheno receives 2 full tide cycles each day and the tidal regime is approximately 6hrs between low and high, with a range of between 0.4m – 1.9m tide heights.

Two control sites and the intertidal and subtidal regions around Abalone Farms Australia were chosen to assess whether the abalone farm effluent impacted the biomass density and species diversity.

4.2.4 Results

4.2.4.1 Dissolved Nutrient Scavengers

4.2.4.1.1 Lower littoral zone

There were greater numbers and percentage cover of *Porphyra columbina* at the impact site compared to the levels exhibited at the control sites on August 2003 and December 2003 (Fig. 4.6) while other sampling periods showed no significant differences. The elevated *P. columbina* numbers at the impact site (August and December 2003) showed ten fold more plants per m^{-2} than the density at the northern control site, and almost 90 fold greater than the southern control site ($P = <0.001$, $F = 17.33$, $n=30$). Similarly the percentage cover of *P. columbina* was approximately ten fold greater at the impact site compared to the northern control site and 50 times greater than at the southern control site ($P = <0.001$, $F = 20.65$, $n=30$) (Fig. 4.7).

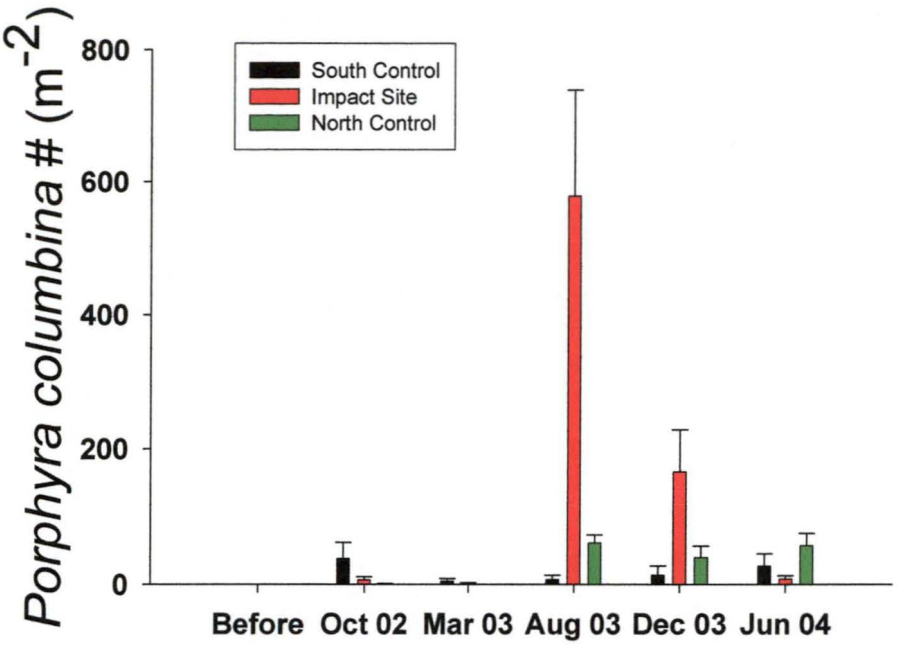

Figure 4.6: Mean *Porphyra* sp. numbers (per m^2) in the lower littoral zone for impact and control sites over time. Absence of bar values indicate absence of species. Before samples taken in July 2002

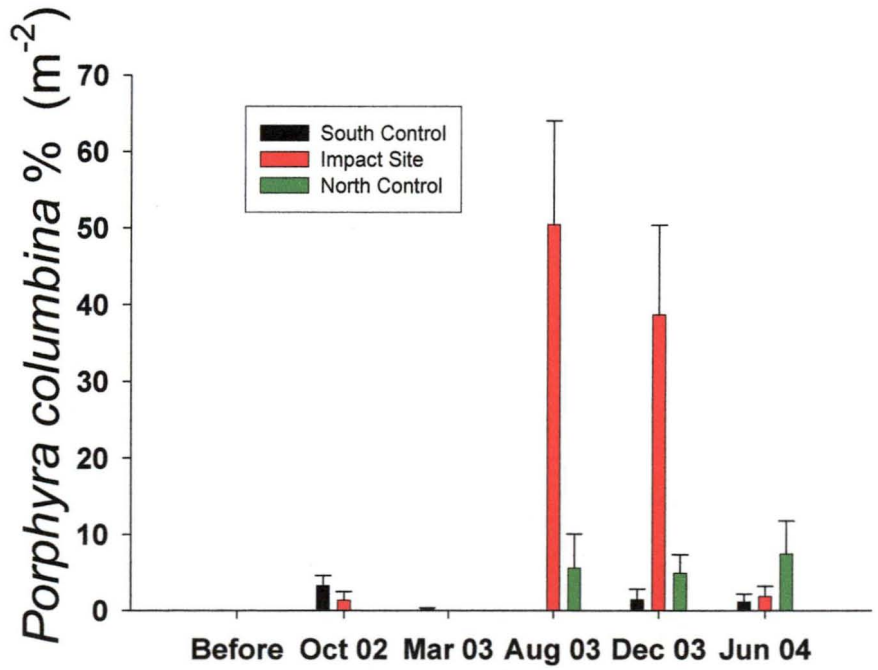

Figure 4.7: Mean *Porphyra* sp. % cover (per m^2) in the lower littoral zone for impact and control sites over time. Absence of bar values indicate absence of species. Before samples taken in July 2002

Over the 2 year sample period *Ulva australis*. showed two peaks in abundance with both occurring during mid spring (Oct 2002) to early summer (Dec 2003). Unlike *P. columbina* however the abundance of *U. australis* at the impact site was similar to the control sites both in pattern and abundance over the period sampled ($P<0.05$) (Fig. 4.8 and 4.9)

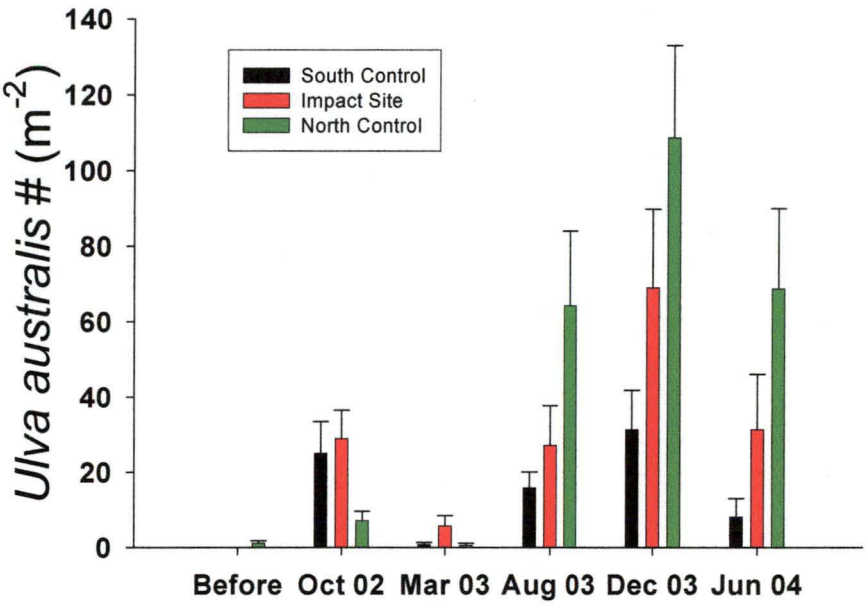

Figure 4.8: Mean *Ulva* sp. numbers (per m^2) in the lower littoral zone for impact and control sites over time. Absence of bar values indicate absence of species. Before samples taken in July 2002

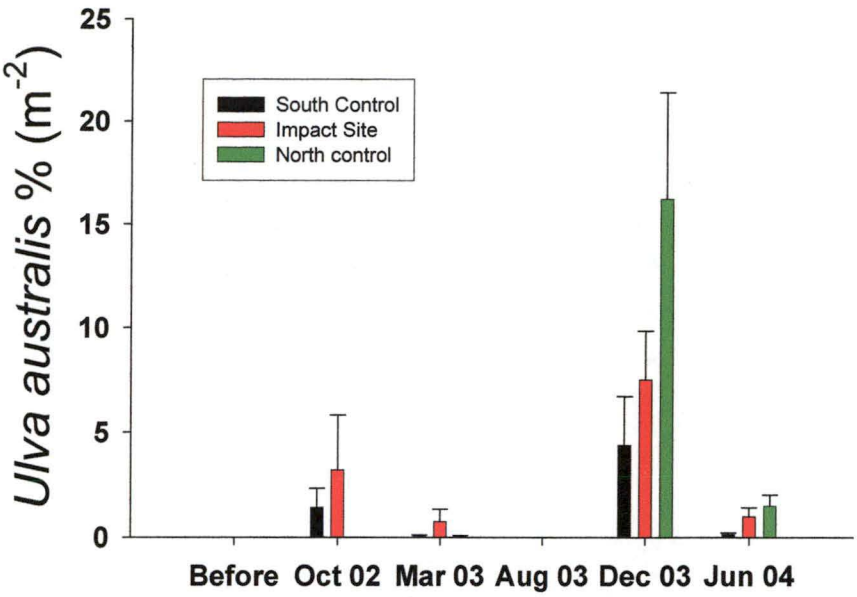

Figure 4.9: Mean *Ulva* sp. % cover (per m^2) in the lower littoral zone for impact and control sites over time. Absence of bar values indicate absence of species. Before samples taken in July 2002

4.2.4.1.2 Upper littoral zone

Porphyra columbina and *U. australis* abundance was significantly lower in the upper littoral zone when compared to the lower littoral ($P<0.05$). There was a high degree of spatial variability in the abundance of nutrient scavenging seaweeds in the upper littoral zone. There was no significant difference in the abundance or percentage cover of *P. columbina* (Fig. 4.10 and 4.11) or *U. australis* (Fig. 4.12 and 4.13) between the impact and control sites for any of the sampling times ($P<0.05$) in the upper littoral zone. Despite this during August 2003 there was much greater mean number of *P. columbina* at the impact site relative to the control sites (Fig. 4.10).

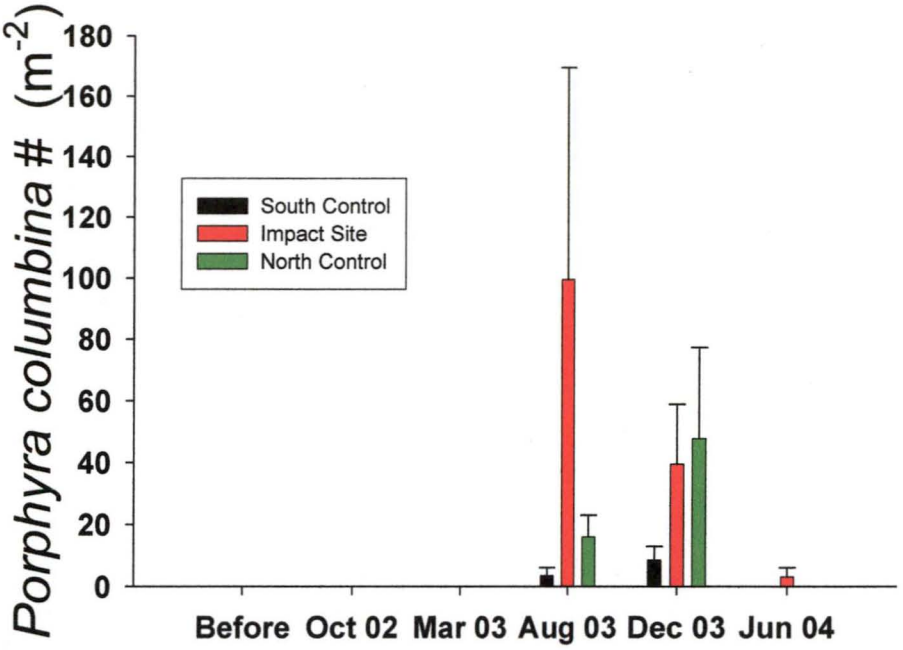

Figure 4.10: Mean *Porphyra* sp. numbers (per m^2) in the upper littoral zone for impact and control sites over time. Absence of bar values indicate absence of species. Before samples taken in July 2002

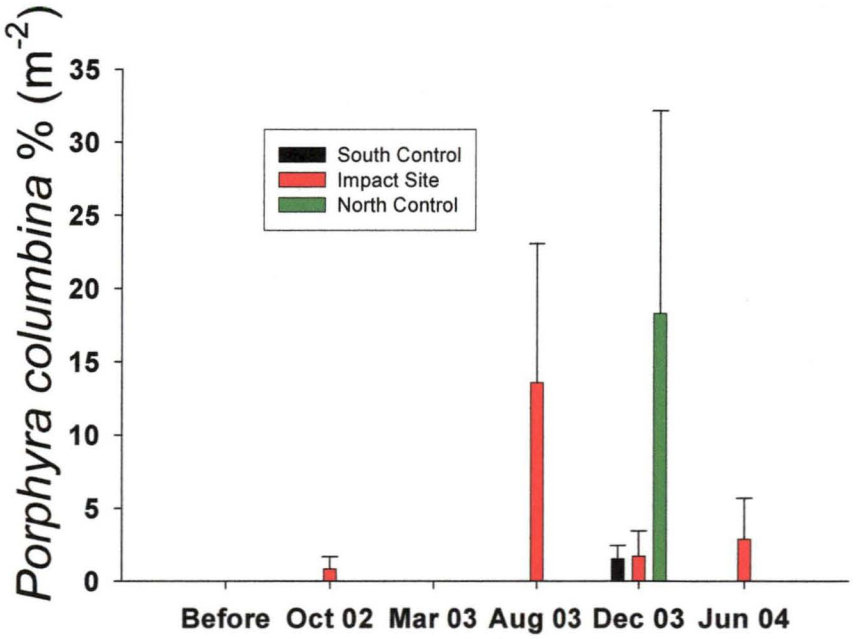

Figure 4.11: Mean *Porphyra* sp. % cover (per m^2) in the upper littoral zone for impact and control sites over time. Absence of bar values indicate absence of species. Before samples taken in July 2002

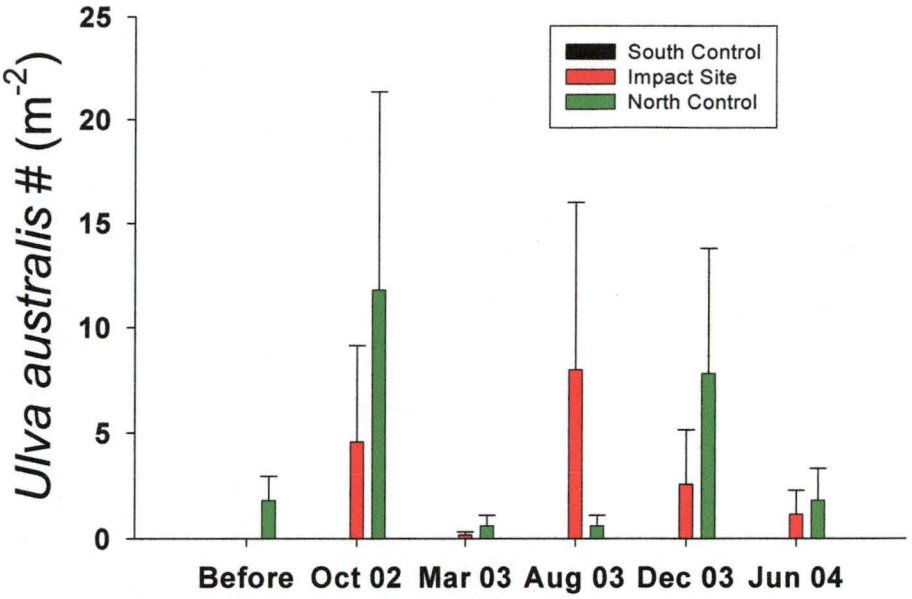

Figure 4.12: Mean *Ulva* sp. numbers (per m^2) in the upper littoral zone for impact and control sites over time. Absence of bar values indicate absence of species. Before samples taken in July 2002

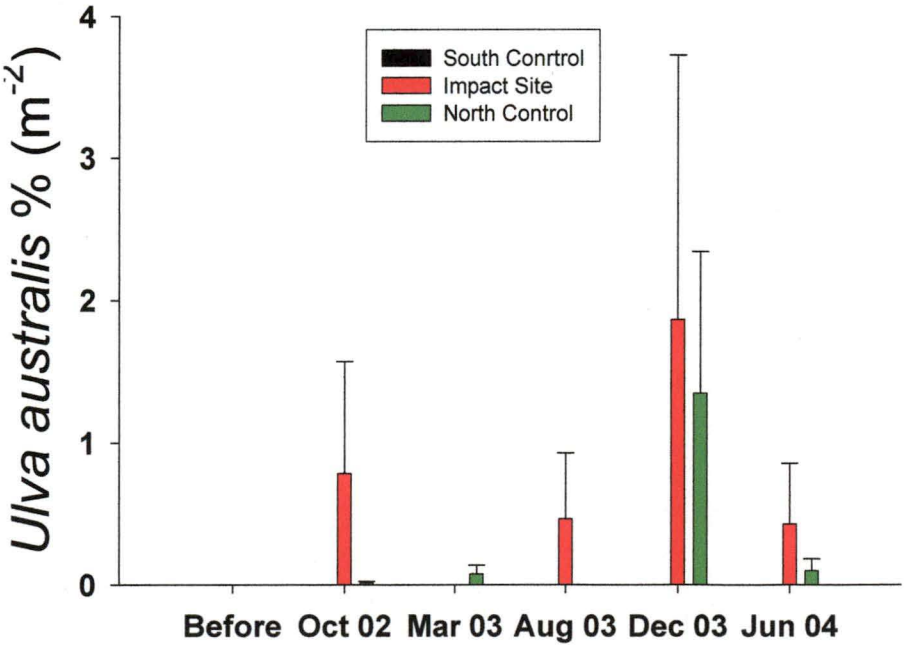

Figure 4.13: Mean *Ulva* sp. % cover (per m^2) in the upper littoral zone for impact and control sites over time. Absence of bar values indicate absence of species. Before samples taken in July 2002

4.2.4.2 Grazers

4.2.4.2.1 Lower littoral zone

The low littoral zone showed no significant differences in Patellid limpet numbers between the impact and control sites ($P = 0.132$, F =2.18, n=29) (Fig. 4.14). The trends shown at the control sites were also exhibited at the impact site with a peak in mean numbers of limpets in October 2002, and subsequent decreases in mean numbers by March 2003.

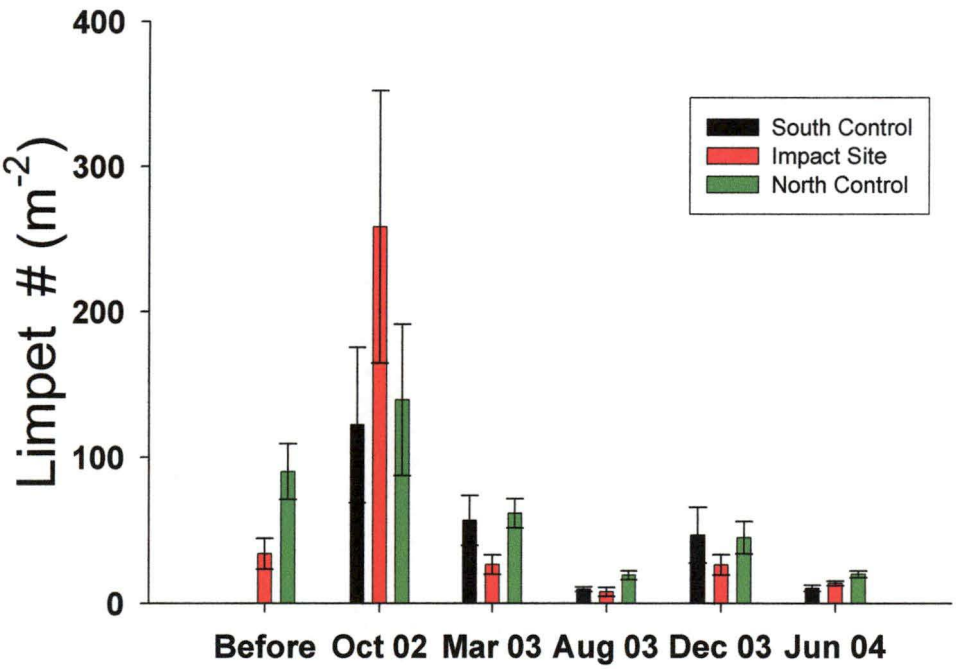

Figure 4.14: Mean number of Patellid limpets per square metre in the lower littoral zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

There was no significant differences in abundance of *Patriella* sp. between the impact and control sites (Fig. 4.15). There was an increase in average *Patriella* sp. numbers at the impact site during August 2003.

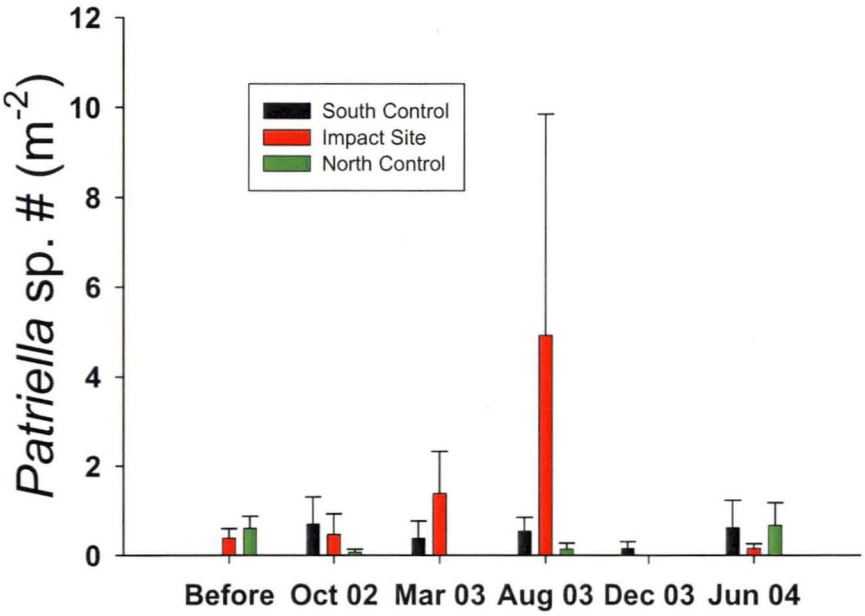

Figure 4.15: Mean number of *Patiriella* sp. per square metre in the lower littoral zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

4.2.4.2.2 Upper littoral zone

In the high littoral zone mean limpet density showed high variability between the control sites and the impact site (Fig. 4.16). There was a degree of consistency between the control sites where the northern and southern control sites showing decreases in the

mean limpet numbers over the course of the sampling. Conversely, at the impact site there was an increase in limpet numbers with a peak in December 03.

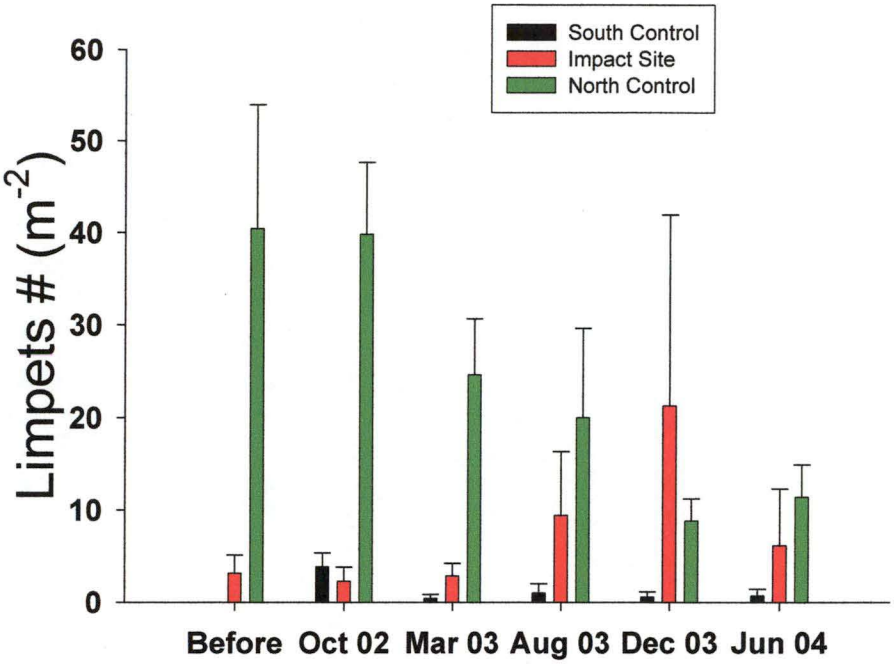

Figure 4.16: Mean number of Patellid limpets per square metre in the upper littoral zone for control and impact sites. Absence of bar values indicate absence of species

Periwinkle *Nodolittorina sp.* (Fig. 4.17) showed no significant deviations from the trends exhibited at the control sites within the high littoral zone. Densities of the Periwinkle *Bembicium nanum* showed no consistent trends between the control and impact sites (Fig. 4.18). . Before samples taken in July 2002

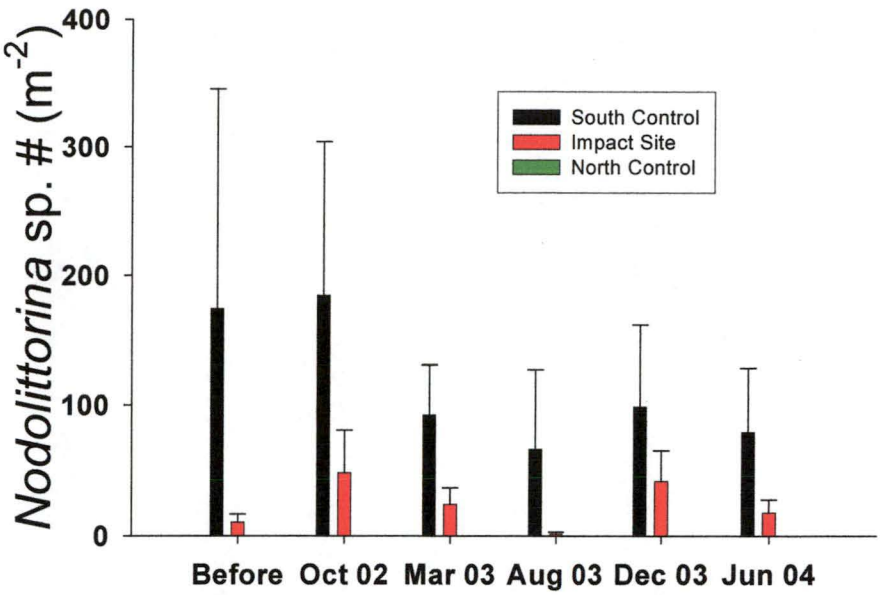

Figure 4.17: Mean number of *Nodolittorina* sp. per square metre in the upper intertidal zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

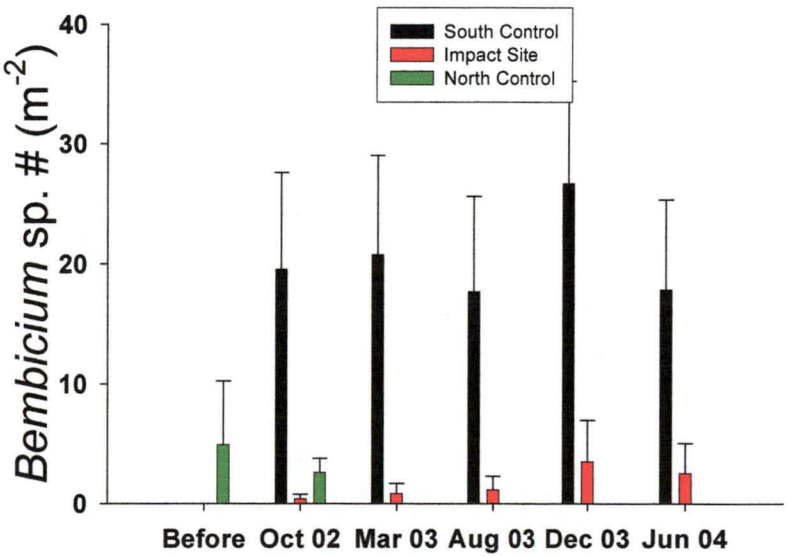

Figure 4.18: Mean number of *Bembicium* sp. per square metre in the upper littoral zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

4.2.4.3 Particulate filter feeders

The upper and lower littoral zones have a number of organisms which are able to capitalise on particulates within the coastal waters such as ascidians, barnacles, bivalves and sponges (Edgar, 2000). Despite no net production of particulates from the abalone farm it is likely there is a change in composition (Chapter 3) and/or reductions in particulates and hence the farm effluent may represent an opportunity for some species to proliferate.

4.2.4.3.1 Lower littoral zone

The temporal trends observed for the barnacle, *Catomerus polymerus,* in the lower littoral zone were similar between the impact and control sites; however lower abundance was exhibited at the impact site during the Aug to Dec 2003 periods ($P<0.05$) (Fig. 4.19). The southern control site showed a peak in the Aug 2003 period with densities similar to the control and impact sites both preceding and following this peak.

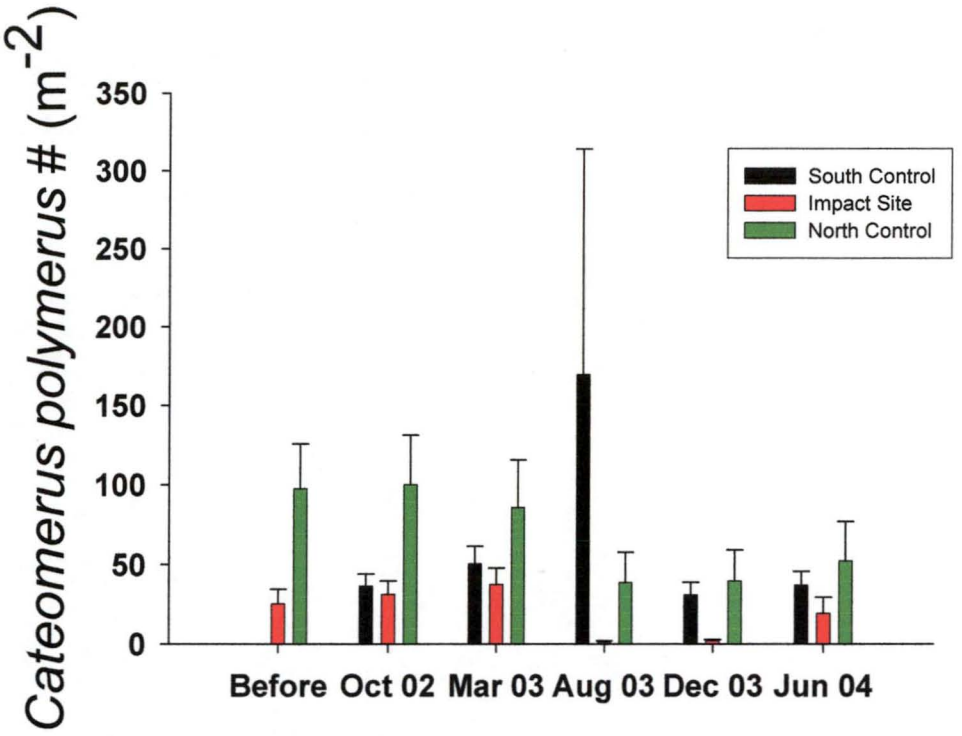

Figure 4.19: Mean number of *Cateomerus polymerus* per square metre in the lower littoral zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

Another filter feeder the intertidal mussel, *Xenostrubus pulex,* was present at relatively low densities up until June 2004 when there were increases at all sites (Fig. 4.20).

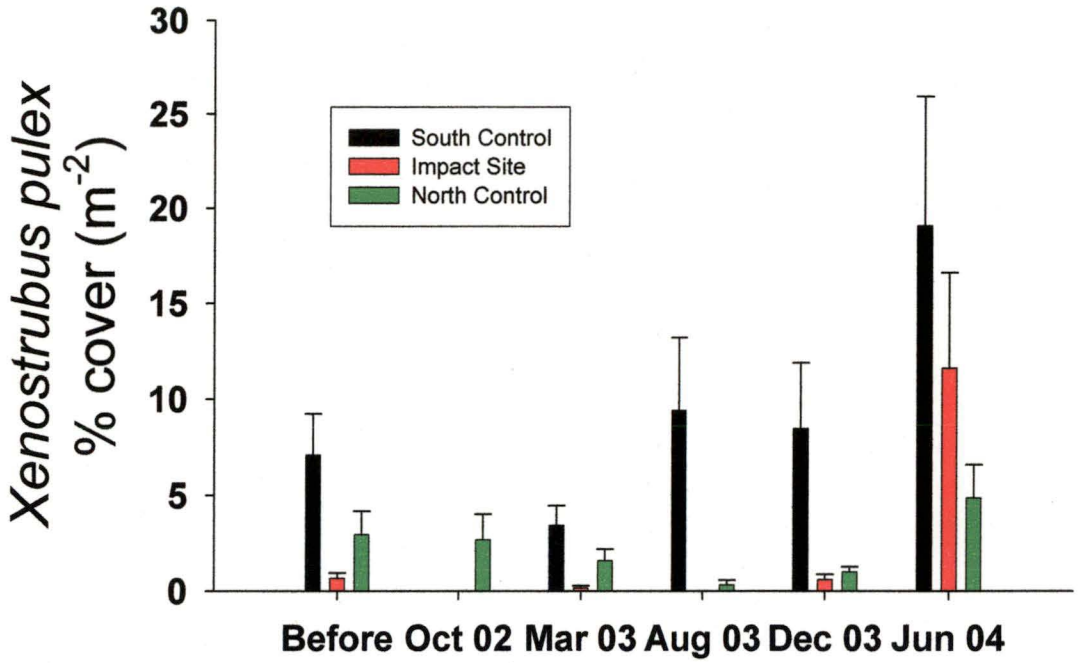

Figure 4.20: Mean number of *Xenostrobus pulex* per square metre in the lower littoral zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

The barnacle, *Chamaesipho tasmanica,* numbers were generally lower at the impact site when compared to the control sites (Fig. 4.21). There was a high degree of spatial variability between the sites, however the trends of a peak in March 03 followed by relatively lower abundances was consistent across sites.

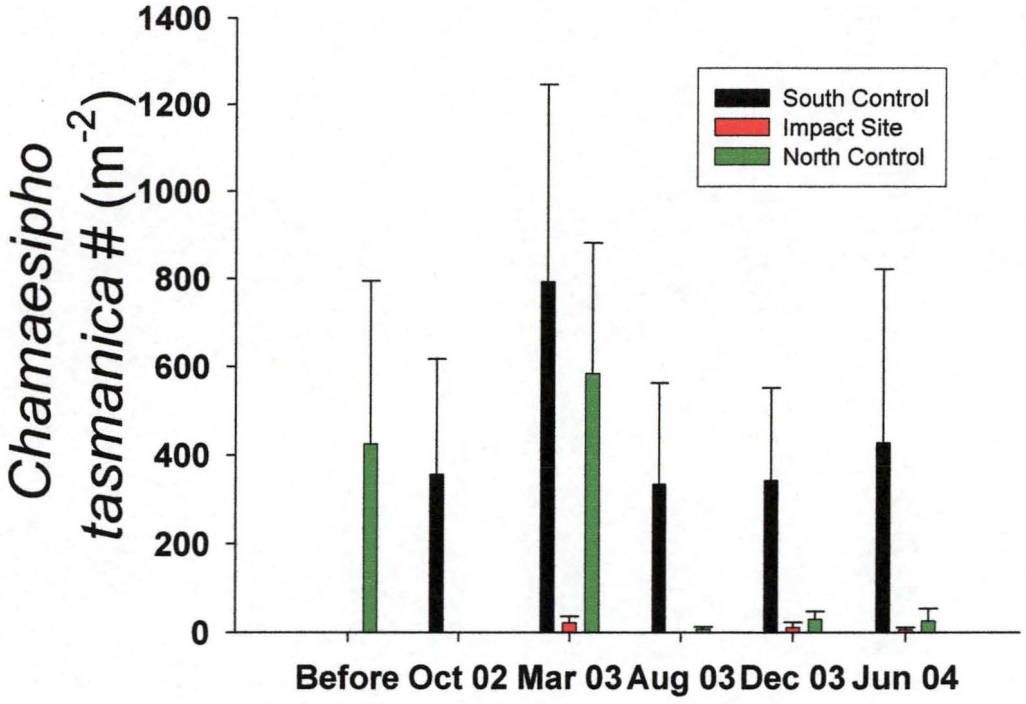

Figure 4.21: Mean number of *Chamaesipho tasmanica* per square metre in the lower littoral zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

4.2.4.3.2 Upper littoral zone

Catomerus polymerus showed similar trends at all sites in the upper littoral zone with a peak in numbers during March 2003 (Fig. 4.22). However over time there was a decrease in the inter site difference.

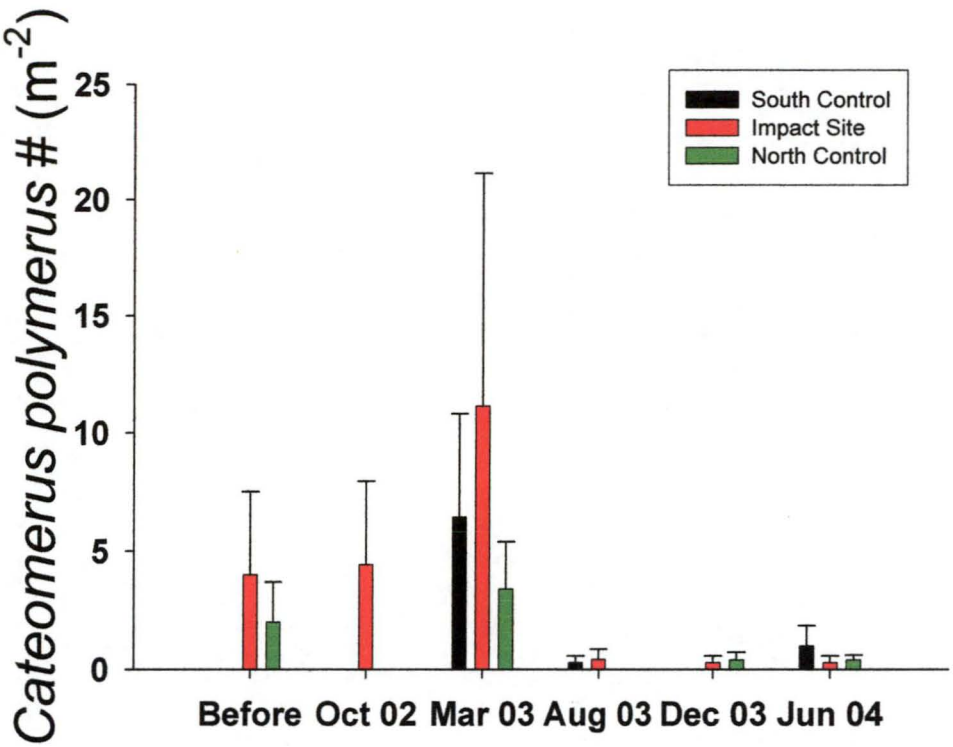

Figure 4.22: Mean number of *Cateomerus polymerus* per square metre in the upper littoral zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

Numbers of the mussel *X. pulex* at the impact site remained relatively low until June 2004 when there was a small increase in numbers (Fig.4.23). The southern control site showed a high degree of spatial variability in *X. pulex* density.

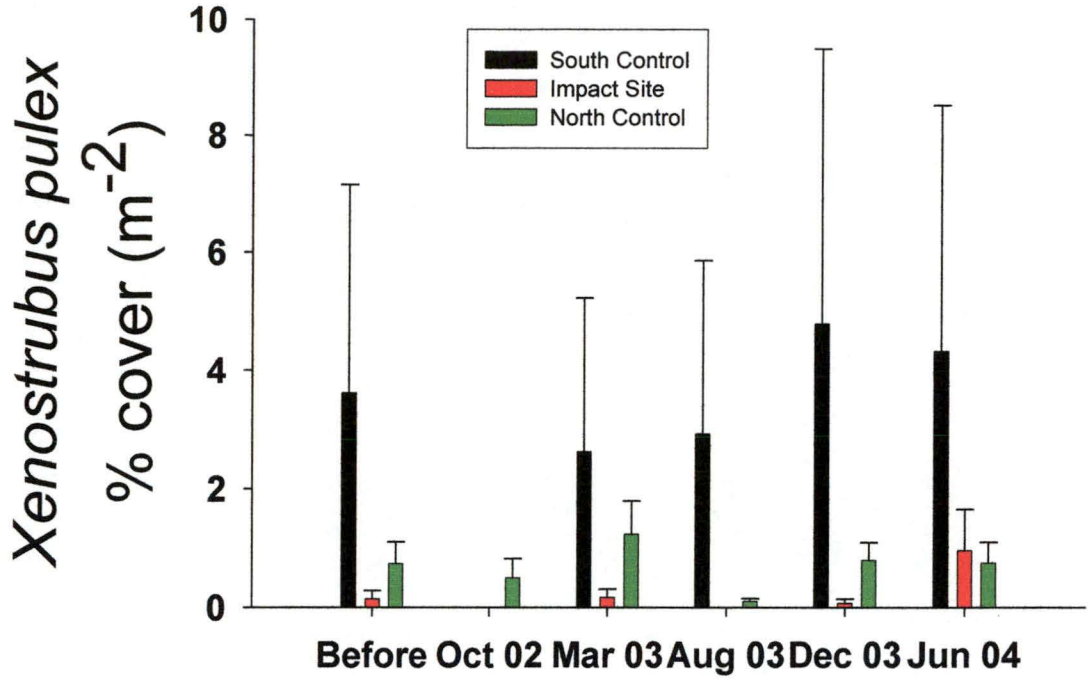

Figure 4.23: Mean number of *Xenostrobus pulex* per square metre in the upper littoral zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

Chamaesipho tasmanica density in the upper littoral zone at the impact site was within the range of the control sites (Fig. 4.24). *Chamaesipho tasmanica* numbers at the northern control site were greater on average than at the impact and south control sites. There were no clear trends evident at any of the sites.

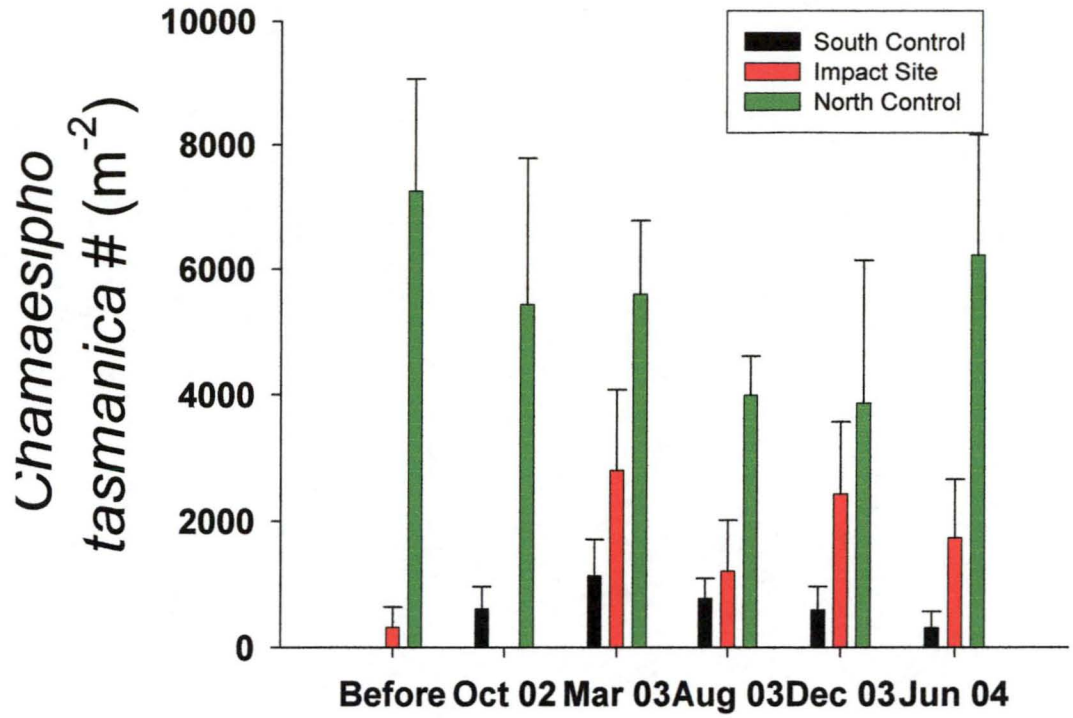

Figure 4.24: Mean number of *Chamaesipho tasmanica* per square metre in the upper littoral zone for control and impact sites. Absence of bar values indicate absence of species. Before samples taken in July 2002

4.2.4.4 Diversity

For the impact site, the trends in Margalef's diversity index were similar to at least one of the control sites for both the upper and lower littoral zones. There appears to be significant intra annual variability that complicates any simple before and after comparison. In the lower littoral zone the temporal trends between all sites appeared to be similar (Fig. 4.25). However for the first sampling period after the farm had begun

operations (Oct 2002) there was a lower mean diversity when compared to the control sites.

In the upper littoral, the impact site was most closely related to the southern control site (Fig. 4.26). For the first three sampling periods the northern control site showed some similarities in trends relative to the impact site, however it appears that there was an increase in diversity during the December 2003 sampling at the northern control site.

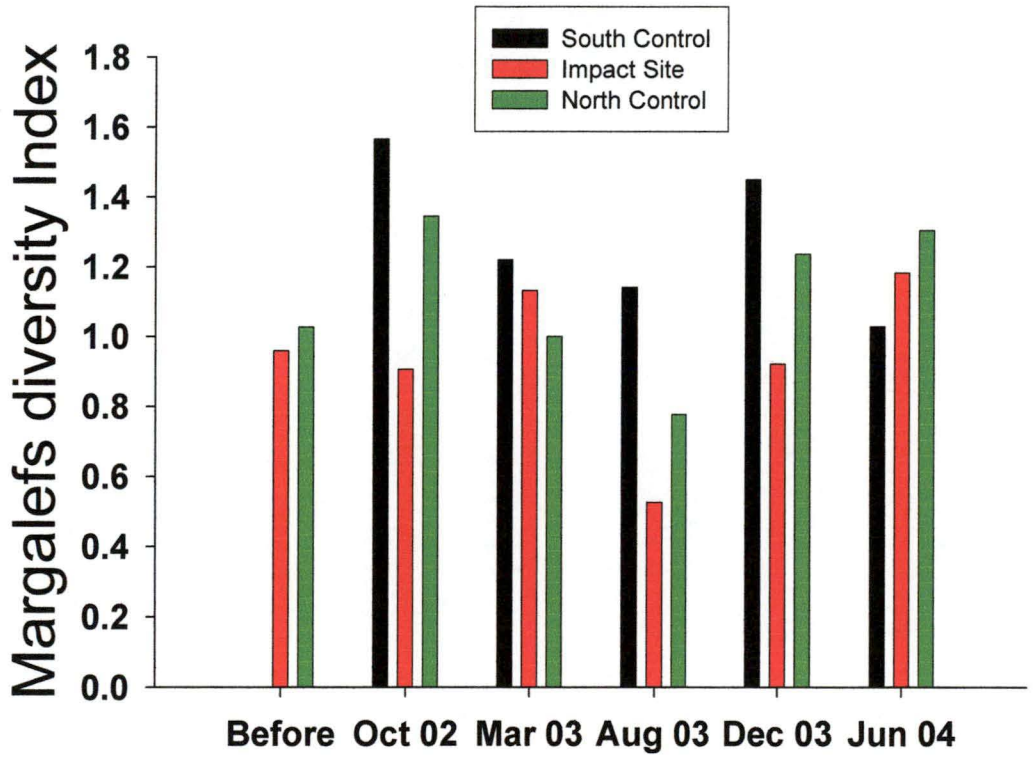

Figure 4.25: Margalefs diversity index for Impact and control sites in the lower littoral region. . Before samples taken in July 2002

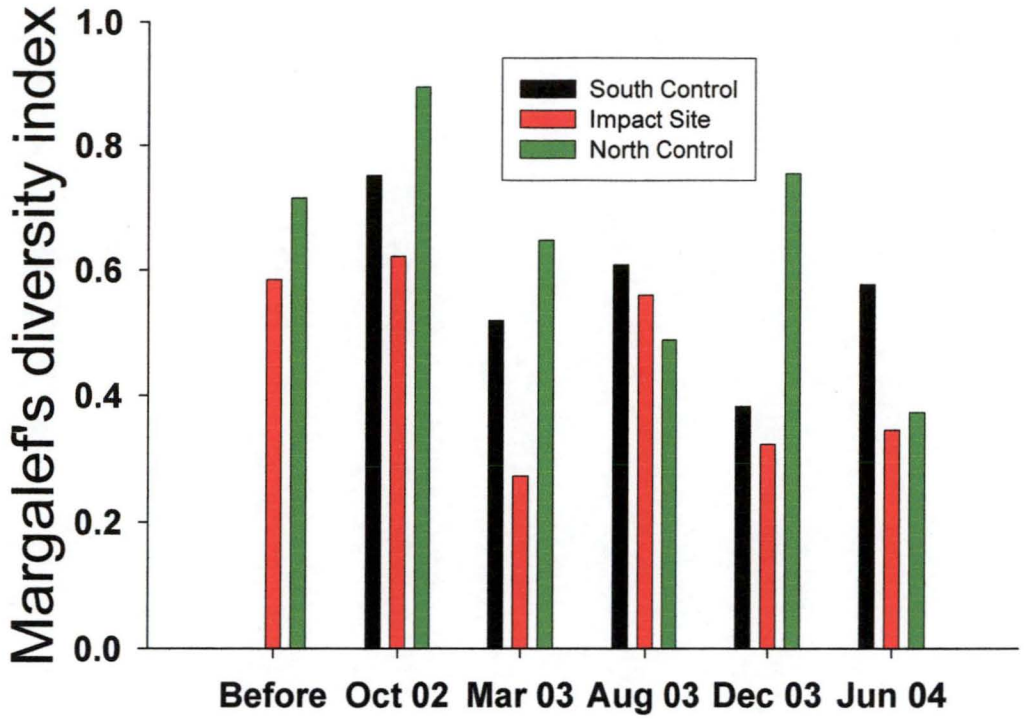

Figure 4.26: Margalefs diversity index for Impact and control sites in the upper littoral region. . Before samples taken in July 2002

4.2.4.5 Transects

The subtidal transects showed similar macroalgal species compositions before and after the farm began operations. Across all sites and times the predominant seaweed was *Phyllospora comosa* which occurred at all distances other than the 10m zone (which was dominated by the bull kelp *Durvillaea potatorum*). Various other species of macroalgae were present however their relative abundances were distinctly lower than for *Phyllospora* and *Durvillaea* species (Table 4.2).

Table 4.2: Species presence and absence at given distances from the end-of-pipe for before (May 2002) and after (June 2004) the farms operations commenced. Grey boxes indicate presence of given species, white boxes indicate absence. Numbers within boxes represent distance (meters) where the species were observed as measured from the end-of-pipe. . Before samples taken in July 2002

Species	North Control		Impact site		South Control	
	Before	After	Before	After	Before	After
Durvillaea potatorum	10	10	10	10	10	10
Cystophora sp.	20	20		20	20	20
Lessonia corrugata	20				30	30
Phyllospora comosa	20,30,40, 50	20,30,40, 50	20,30,40, 50	20,30,40, 50	20,30,40, 50	20,30,40, 50
Unidentified Red algae	10	10				

4.2.5 Discussion

4.2.5.1 Dissolved Nutrient Scavengers

Typically around point-source discharges delivering nutrients to the environment (such as sewage outfalls), there is strong growth of opportunistic macroalgal species (i.e. R-strategists such as *Ulva* sp., *Gelidium* sp., *Colpomenia* sp. *Enteromorpha* sp.) (Ashton and Richardson, 1995; Dhargalkar, 1986; Terlizzi et al., 2002; Underwood and Chapman, 1996), while decreasing the abundance of larger brown macrophytes (commonly K-strategists) such as *Homosaria banksii* and *Durvillaea potatorum* (Doblin and Clayton, 1995), Sargassum agardhianum (Littler and Murray, 1975), and decreased diversity surrounding the outfall (Fairweather, 1990; Littler and Murray, 1975; Roberts, 1996;

Smith, 1996b; Underwood and Chapman, 1996). The results for Abalone Farms Australia suggests an increase in *Porphyra columbina* and possibly *Ulva australis* populations within the intertidal region surrounding the end-of-pipe, a trait common for enriched water bodies (Chopin et al., 1999; Malta et al., 2002; Pedersen et al., 2004; Raffaelli et al., 1998). Specifically AFA was likely to impact the lower littoral region more heavily than the upper littoral region with respect to nutrient scavenging *P. columbina.* In the lower littoral zone % cover of *P. columbina* were up to 10-50 orders of magnitude greater at the impact site than at the control sites; however, these increases were not consistent through time. While this study did not determine the reasons for the seasonal variability, the increase in *P. columbina,* occurred primarily around spring time in both 2002 and 2003. At all other sampling times the density of *P. columbina* was relatively lower. During the summer months the decrease in *P columbina* densities was perhaps due to the increased desiccation associated with summer periods (Mizuno, 1984) which was further exacerbated by the high surface area to volume ratio of *P. columbina* (Lobban and Harrison, 1994). Alternatively *Ulva australis,* the other observed nutrient scavenging seaweed, showed a later peak in density during the beginning of summer. That *U. australis* was able to show a greater tolerance to desiccation was perhaps due to the ability of *U. australis* to form dense mats (Tanner, 1986) and thus hold more water through decreased surface area to volume ratios (Lobban and Harrison, 1994). Eventually at all sites the greater degree of desiccation and evaporation during the summer months is likely to cause the reduction of ephemeral and desiccation intolerant seaweeds to very low numbers in the upper littoral and to a lesser extent the lower littoral zones (Raffaelli and Hawkins, 1996). Despite this the reduction in abundance of *P. columbina* and *U.*

australis is not solely dependent upon desiccation and temperature, the role of grazers will be discussed later.

Another point of interest is the much smaller peak in abundance of dissolved nutrient scavengers during the spring of 2002 compared to the spring of 2003. This is likely an effect of the nutrient sources. During 2003 the total farm effluent ammonium concentrations began increasing well above ambient concentrations (Chapter 3) and hence are likely to drive the increases in nutrient scavenging seaweed biomass. The data suggests that *Porphyra* sp. with a mean count of up to 620 individuals per m^2 (in the low littoral region), were most effective in utilising the nutrients from the farm (when compared with *Ulva* sp. - count = $63/m^{-2}$). This information suggests that if a relationship between effluent ammonium loads and *P. columbina* mass were established, potentially *P. columbina* could be used as a bio-indicator. Additionally the information suggests that examination of the intertidal region would be best conducted during late winter to early spring for the capture of the peak seaweed abundance. However further work is required before this species could be used reliably. In particular the growth of *P. columbina.* in the lower littoral region appeared to be more substantially influenced by the effluent relative to the upper littoral region. It is likely that the lower littoral provided a more optimal environment relative to the upper littoral in terms of the physical factors which affect growth (temperature, desiccation, water motion (Lobban and Harrison, 1994)).

4.2.5.2 Grazers

There does not appear to be any clear effect of the abalone farm on the intertidal grazing population surrounding the end-of-pipe. With respect to grazers, the impact and southern control site were more closely related in terms of seasonal variation in numbers of individuals.

There is evidence to suggest that in nutrient rich bodies of water grazing communities can respond by either increasing or decreasing in abundance. Given that limpets are the dominant grazers within the intertidal region (Jenkins and Hartnoll, 2001; Underwood, 1979) and their abundance can be related to the seasonal supply of epilithic algae (Jenkins and Hartnoll, 2001); an increase in the grazing community surrounding the outfall is a plausible hypothesis given that the abalone farm exports microalgae and bacteria to the marine environment (Appendix). A significant increase in limpets was not observed and may have been unmeasured due to the large increase in *P. columbina,* which in many cases accounted for 100% of the quadrat area. This may have been physically masking other species (i.e. limpets, starfish) in the understorey. When checks were made species of limpets were found in the understorey, however the time consuming nature of counting the understorey, the number of quadrats, the short window to be able to make the counts (i.e. low tide period) resulted in only counts of the canopy being conducted.

There is also evidence to suggest that macroalgal canopies can affect competition for space in the intertidal region and hence suppress the abundance of some grazing species. Under some conditions (particularly in the lower littoral region) foliose algae is able to grow faster than the grazers can consume them (Rosemond, 1996; Vanni, 1996).

Hence the bare rock space required for grazing may be reduced therefore reducing the recruitment of limpets (Raffaelli and Hawkins, 1996). This scenario, however, could only occur after the macroalgal canopy has been fully established to the point where grazing by limpets is unlikely to significantly reduce abundance (Raffaelli and Hawkins, 1996).

As summer approaches, macroalgae abundance is likely to be reduced due to desiccation (as described above) and hence the conditions may favour limpets which are able to compete more effectively for intertidal space (Underwood and Jernakoff, 1984). However as the severity of the summer conditions increase in the upper littoral zone and macroalgae density continues to decrease, the limpets themselves may begin to experience conditions which are unfavourable (i.e. lack of food and desiccation) and this may account for the decreases in abundance seen post Dec 2003 (Raffaelli and Hawkins, 1996).

Patiriella sp. did not show statistically significant increases in abundance in the lower littoral regions during the Mar 2003 – Aug 2003 period. Species within the class Asteriodea are capable of scavenging upon a variety of plant and animal material that is locally available (Edgar, 2000). Specifically *Patiriella calcar* is omnivorous and may be capable of feeding upon algae, bacteria and detritus (Arrontes and Underwood, 1991; Edgar, 2000). The abalone farm effluent indirectly and directly provides at least two of these food sources. Elevated levels of algal growth supported by the increased ammonium concentrations from the outfall, combined with the elevated bacterial content of aquaculture effluents (Erler et al., 2004; Hargreaves, 1998; Moriarty, 1997) may be allowing the observed increase in mean *Patiriella* numbers during August 2003.

4.2.5.3 Particulate filter feeders

Over the period sampled the majority of times saw no net release of particulates from the abalone farm (Chapter 3). The lack of a significant particle load is consistent with no change in the density of particulate filter feeding animals at the impact site relative to the control sites. This is true for both the upper and lower littoral zones for *Chamaesipho tasmanica, Xenostrubus pulex, and Catomerus polymerus*. Despite this, there is some evidence of occasions where there was an increased organic content of the effluent particulates (relative to farm inflow – Chapter 3) and hence future monitoring may be warranted. Certainly it would seem likely that farms without sedimentation ponds would experience, at least seasonal, increases in filter feeder abundances surrounding their outfalls.

While no detectable changes other than the proliferation of nutrient scavenging seaweeds occurred, there may be a number of properties of the effluent which may potentially negatively influence biota surrounding the outfall. A possible effect of the abalone farm on the intertidal grazing and filter feeding population is the toxic effect of ammonium and/or nitrite (Russo, 1985). Studies have shown that for bivalve molluscs as little as 10μm ammonium concentrations can decrease growth rates and 235μm will have a lethal effect (Epifanio and Srna, 1975) and for univalve abalone under 2.5-3.5μm is likely to cause significant growth reductions (Colt and Armstrong, 1981; Harris et al., 1998) (AFA outflow maximum concentration = 10μm ammonium). Therefore despite not seeing any evidence of reductions in biomass of grazing and filter feeding species, there is likely to be an effect of the ammonium in the effluent on these populations surrounding the outfall.

4.2.5.4 Diversity

The decline in diversity (at the impact site) for some months of the year in both the lower and upper littoral zones is likely due to the proliferation of nutrient scavenging seaweeds which covered up to 100% of the available surface area in some of the quadrats. Such dominance of the seaweeds would have the effect of reducing the recorded diversity within the intertidal regions simply due to the masking of understorey species. The return of the impact site diversity to a level similar to the control sites is likely due to the reduction in nutrient scavenging seaweeds covering the quadrats and hence some of the species within the understorey may be visible for counting again.

4.2.5.5 Transects

Transects before and after the commencement of discharge at the impact and control sites offer an insight into the effects of abalone farming on the marine environment. The qualitative nature of the study was unavoidable due to the time consuming nature of performing underwater quadrats and also the shortage of fully qualified volunteers required for diving. The information gained from the study shows that macroalgal species diversity within each of the sites was similar in the before and after periods. Despite this it should be noted that this study only measured the macroalgal canopy and not the understorey. Therefore it is possible that some species of the understorey may have been impacted and that this was not detected.

With respect to actual abundance of each of the species, while no quadrats were taken, the video footage suggests the abundance of each species of algae was similar for both the before and after periods at the impact site. *Durvillaea* sp. beds formed the

predominant seaweed in the low littoral and subtidal region, and *Phyllospora* sp. dominated in the subtidal zone (Appendix). Potentially the technique of video transects was not sensitive enough to detect the changes in macro algae which may be occurring in the subtidal region. Another possibility is that change in the dominant and relatively long lived species may take longer to manifest itself. Perhaps a more suitable technique would have been to examine growth rate related changes such as RNA: Protein ratios or N^{15} isotope uptake.

4.2.6 Conclusions

Overall there was a high degree of natural spatial and temporal variation in the intertidal regions surveyed with respect to the intertidal community. In all cases, despite a proliferation of some of the species within each group, there was a return to near baseline conditions (densities similar to control sites) for all species at some time of the year when the conditions became unfavourable for growth and reproduction (i.e. summer).

In terms of community shift, the abalone farm is likely to be contributing to the increase in the nutrient scavenging macroalgae *P. columbina*. The dominant species of particulate filter feeding organisms and their abundances were variable such that differences relative to control sites were inconsistent. In terms of species diversity there were reductions in diversity at some times of the year specifically with the early spring proliferation of the *P. columbina*, however diversity was maintained over the period of sampling for the similar seasons of the year and no discernable trends towards losses or gains were detectable at the impact or control sites..

CHAPTER 5: Minimisation of Abalone Farms

Australia's effluent nutrients

5.1 Introduction

Within Australia there is the need to reduce the loads of nutrients entering the coastal waterways to prevent eutrophication and ensure the sustainable use of our marine environment. Commonly reductions in nutrient discharge by industries are associated with increased operational costs and may have limited direct financial benefit (aside from increased business through public perceptions). Means of reducing effluent nutrients which also increase profitability are of great use to industry and should be explored wherever possible. In chapter 1 it was demonstrated that within an abalone farm the formulated feed entering the water was responsible for the increased nutrient load delivered to the coastal environment. While many aquaculture facilities view these nutrients as a waste, in the case of abalone farms they are actually a resource which is capable of reducing operating costs. This may be achieved through the culture of algae which may both reduce effluent nutrients and also provide a supplemental food source . (Neori et al., 2003; Neori et al., 1998; Neori et al., 2000). Further to reducing operating costs the seaweeds may in fact increase the health of the abalone by supplying essential vitamins and minerals; components which may leach rapidly from formulated feeds (Mai, 1998).

Surrounding outfalls which input nutrients into the marine environment, a number of opportunistic fast growing macroalgal species can compete very effectively for light and nutrients (Rivers and Peckol, 1995). Potential candidates for utilising these nutrients include *Ulva* sp. (Ashton and Richardson, 1995; Campbell, 1999; Fairweather, 1990; Smith, 1996b), *Porphyra* sp (Chopin et al., 1999; Wheeler and Bjornsater, 1992),

Enteromorpha sp. (Fairweather, 1990; Martins and Marques, 2002; Reddy et al., 1992) and some diatoms species (Harding, 1994; Piehler et al., 2004). These algae are eaten by most abalone species (Shepherd, 1975) and further it has been shown that equal growth rates can be achieved for abalone fed enriched *Ulva* sp. and fed formulated feed (Boarder and Shpigel, 2001). In addition to this Neori et al. have developed numerous integrated systems which culture seaweed, fish and abalone in a polyculture system (Neori et al., 1991; Neori et al., 1996; Neori et al., 2003; Neori et al., 1998; Neori et al., 2000). However, despite the potential benefit to growth rates, waste minimisation and other advantages of culturing abalone and *Ulva* sp. simultaneously, grown in this manner seaweeds are only ever likely to be a supplement to the formulated feed. This is primarily due to the fact that in a polyculture system the formulated feed is the source of most of the nutrients required for the growth of the seaweeds.

Another advantage of seaweed and abalone co culture is the potential threat of formulated feed shortages. This is an issue raised amongst farmers in Tasmania (Tasmanian Abalone Growers Association meeting, 2003). While no farm has recorded any problems with formulated feed supply to date, potentially a shortage could occur as all feed enters Tasmania by shipping. If union strikes or shipping problems were to occur, seaweeds may supply an interim amount of food for the animals.

Other researchers have used effluent water to culture species such as *Ulva* sp. in an unattached form within separate culture tanks (Cohen and Neori, 1991; Del Rio et al., 1994; Lekang et al., 2000; Neori et al., 1991; Neori et al., 1996; Neori et al., 2003; Neori et al., 1998; Porrello et al., 2003; Shpigel and Neori, 1996). These studies have shown that integrated systems can remove anywhere between 3.1- >90% of total nitrogen found

in the effluent from the abalone culture system. Typically these systems have very high aeration to keep the unattached seaweeds in suspension and to increase nutrient and gas exchange. While such systems allow the manipulation of conditions to suit the seaweed and hence maximise the uptake of nutrients, they also represent an added infrastructure cost. Ideally it would be best to grow the seaweeds within the same culture tanks as the abalone (i.e. the deep water style tanks used in abalone culture in Tasmania are ideal for this purpose) thus utilising the tank water column and not incurring extra infrastructure cost to the farmers. If this is to occur a means of separating the abalone and the seaweeds is needed to ensure the seaweeds may be able to proliferate and uptake nutrients without excessive grazing pressure. One means of achieving this is to attach seaweeds to a substrate which floats on the surface of the tanks (i.e. seaweed rafts). These rafts serve to keep the seaweed away from the abalone and also acts as a shading and insulation barrier during the daytime hours.

The present study examines the effect of culturing attached seaweed sp. on the nutrient concentrations of the tank effluent. Due to the variability in the seaweed rafts, the availability of tanks and the day to day variability in the feeding performance of different tanks, the rafts could not be tested within the tanks used for commercial production of abalone. Subsequently an experimental system (Fig. 5.1) was designed to test the seaweed rafts capacity to reduce effluent nutrients.

5.2 Materials and Methods

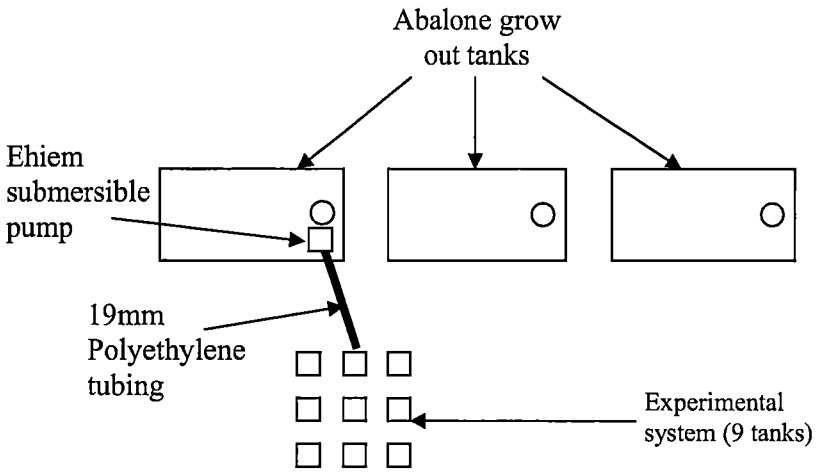

Figure 5.1: Schematic of the experimental system used to test the ability of the seaweed rafts to reduce grow out tank effluent nutrients.

Three commercial grow-out abalone tanks were chosen to monitor for effluent nutrients and effluent water column particulate matter over a 24 hour period. Conditions in each of these grow-out tanks were representative of others on the farm with respect to water flow rates (25L/min), stocking density, feeding rates, aeration rates and surface area. From these three grow-out tanks one tank was randomly chosen to deliver water to the experimental system, while the other two tanks were monitored for effluent nutrients from the tank outflow at two hourly intervals for 24 hours. In the randomly chosen tank an Eheim™ pump was placed near the tank outflow. Through this pump water was delivered (via 13mm clear polypipe) to an experimental system. This experimental system was designed to test the seaweed rafts ability to reduce nutrient concentrations

under simulated grow out tank conditions. A series of nine 40L fibreglass tanks were laid out on the base of an empty concrete abalone tank. The water was delivered to the 9 tank experimental system via 13mm clear polyethylene tubing. Aeration was set consistently amongst all experimental tanks and at a rate equivalent to the grow-out tanks (set by visual assessment). Water inflow rates into the eight tanks of the experimental system were adjusted to between 360mls and 400mls/min/tank and the one additional tank was used to control excess water pressure and collect inflowing water samples. Water flow rates and hence residence times in the experimental system did not replicate flow rates in the grow-out tanks. The residence times in the experimental system were more than three times shorter than in the commercial grow-out tanks. The reason for this was primarily due to the sampling regime and the difficulty of setting the flow rates. The flow rates were initially targeted to be set at 0.120L/min ± 10%, however this was a concern as the volume of water needed from each tank for sample analysis was approximately 1.2L (600mls for TSS, 600mls for nutrients). Therefore the time taken to complete one period of sampling at a water flow rate of 0.120L/min (i.e. sample all tanks within the experimental system and recording of other parameters) would have been in excess of an hour and a half. The other point of consideration was the accuracy of setting the flow rates; a factor that can affect nutrient concentrations. Adjustment of the taps to achieve an even flow rate across all tanks was virtually impossible when the target was 0.120L/min ± 10%. More consistent values within 10% were achieved when this figure was increased three fold.

A seaweed raft (dimensions = 6.4m x 2.3m) exhibiting uniform macroalgae coverage and density was then chosen from a single commercial culture tank (a total of 7

rafts growing on the entire farm). From this raft sections were cut to make smaller seaweed rafts (40 x 40cm, plastic base + seaweed had an average weight of 316 ± 25g SE). The smaller seaweed rafts were then randomly allocated to 4 of the 8 experimental tanks, and left for 24 hours prior to beginning the experiment. This allowed for the acclimation of the seaweed rafts to the conditions within the experimental system.

Five hours prior to the commencement of the experiment the grow-out tanks were cleaned and flushed of all faeces and uneaten feed as per normal practice. A known weight of Adam and Amos artificial 1mm commercial diet, equivalent to a normal daily ration was then fed to the animals at approximately 3:30pm.

Subsequently, at two hourly intervals, triplicate water samples for nutrients and water column TSS were taken from each of the experimental tank's outflows. The water system inflow to the experimental system was also sampled for the above parameters. Additionally light measurements above each experimental tank (approximately 5cm above the waters surface in the centre of the tank) were taken using a Biospherical Instruments QSL100™ light meter. To measure temperature and dissolved oxygen, a YSI sonde 6600 data logger was used and placed in one of the 40L experimental system tanks and set to record every 3 minutes. Approximately every two hours the data logger was switched from a tank with a raft to a tank without a raft. The data logger was alternated between the same 2 tanks each time to ensure any trends exhibited over the sampling period were a function of the difference between the two treatments and not inter-tank differences.

Nutrients analysed included, ammonium, nitrite, nitrate, silicate phosphate and total nitrogen. All analysis was conducted on a Technicon® AAII autoanalyser as

outlined in (Plaschke, 1999), except for ammonium which was analysed by the methods described in (Watson et al., 2004). TSS was conducted according to the methods in (Chapter 3).

Statistical analysis was conducted using paired t-test where each sample was deemed independent through time. This was due to the short residence time and complete exchange in all tanks between the 2 hourly sampling periods.

5.3 Results

The results indicate that the seaweed rafts were effective in reducing some of the measured nutrients within the abalone farm effluent. Figure 5.2 shows a clear difference ($P < 0.05$) in ammonium concentration between tanks which contained seaweed raft treatment; and both the system inflow water and the no seaweed raft treatment. In the majority of cases there was no significant difference between the experimental system inflow water and the tanks having no seaweed rafts.

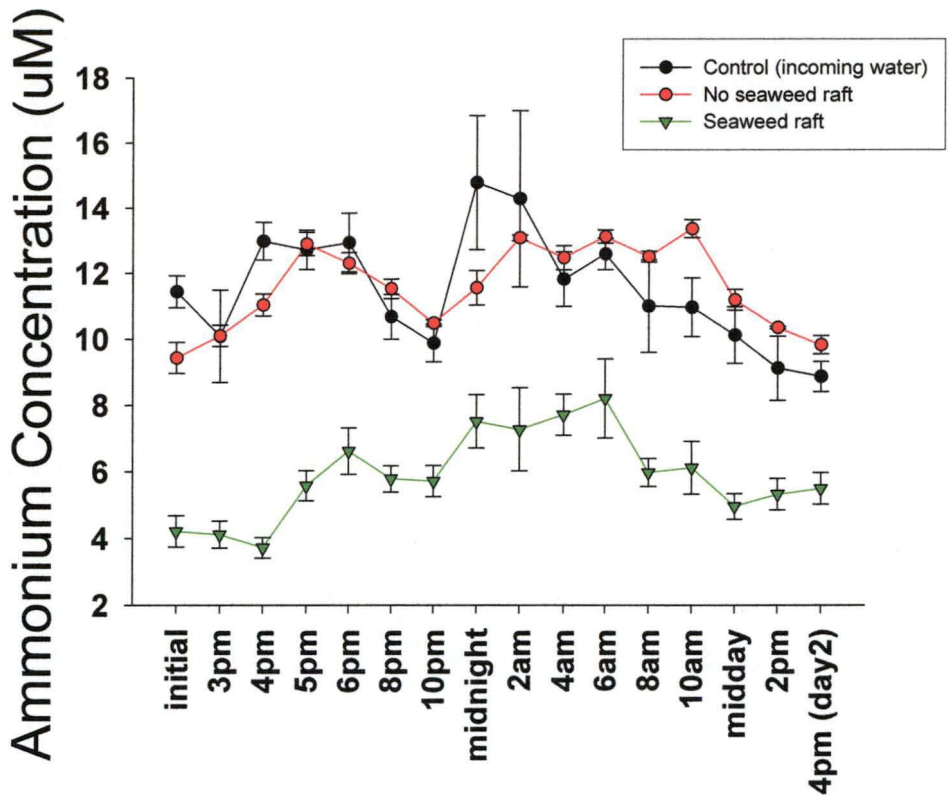

Figure 5.2: Ammonium concentration for system inflow control water, effluent tank water without seaweed raft and with seaweed raft (control $n = 3$, treatments $n = 4$ mean ± SE).

The capacity of the seaweed raft to uptake ammonium was inconsistent over the 24 hour sampling period with the seaweed reducing ammonium concentrations by between 1.4-3.0 µM per 100g of wet seaweed weight or between 34-71% of the inflow ammonium concentration. Some of this temporal variation may be attributed to the variation in inflow ammonium concentration over time as a correlation exists between the inflow ammonium concentration and the amount of ammonium taken up per 100grams of wet algal tissue (Pearsons R = 0.742, n=16, P=0.001). Light intensity showed little effect

on the capacity of the seaweed to reduce ammonium with no correlation existing between uptake and irradiance (Pearsons R = 0.258, n=15, P= 0.354).

Seaweed rafts showed little effect on the silicate concentrations with no statistically significant differences between treatments seaweed raft and no seaweed raft treatments (Fig. 5.3). All treatments showed similar trends with a peak in silicate concentrations between 4-8am.

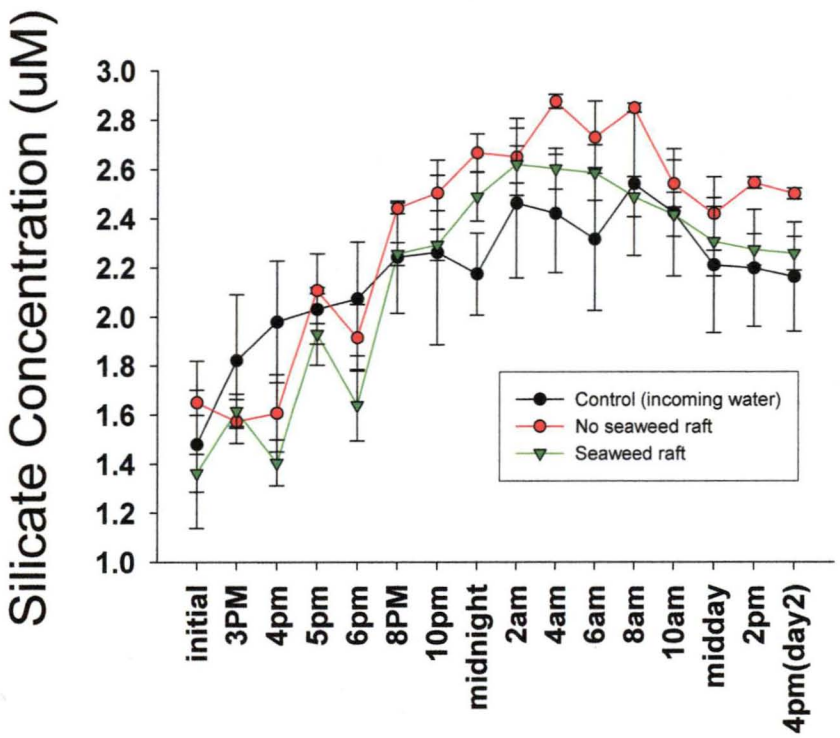

Figure 5.3: Silicate concentration for system inflow control water, effluent tank water without seaweed raft and with seaweed raft (control n = 3, treatments n = 4 mean ± SE).

Phosphate concentrations were marginally reduced (P < 0.5) by the seaweed rafts at various times over the 24 hour sampling period (Fig. 5.4). While the seaweed raft

treatment and system inflow phosphate concentrations showed similar patterns, for all cases the seaweed raft treatment showed lower mean concentrations of phosphate when compared with the system inflow water and the no seaweed raft treatments. A sharp peak in concentration was exhibited at 6pm after which the concentrations rapidly decreased followed by a slow reduction over the duration of the sampling period.

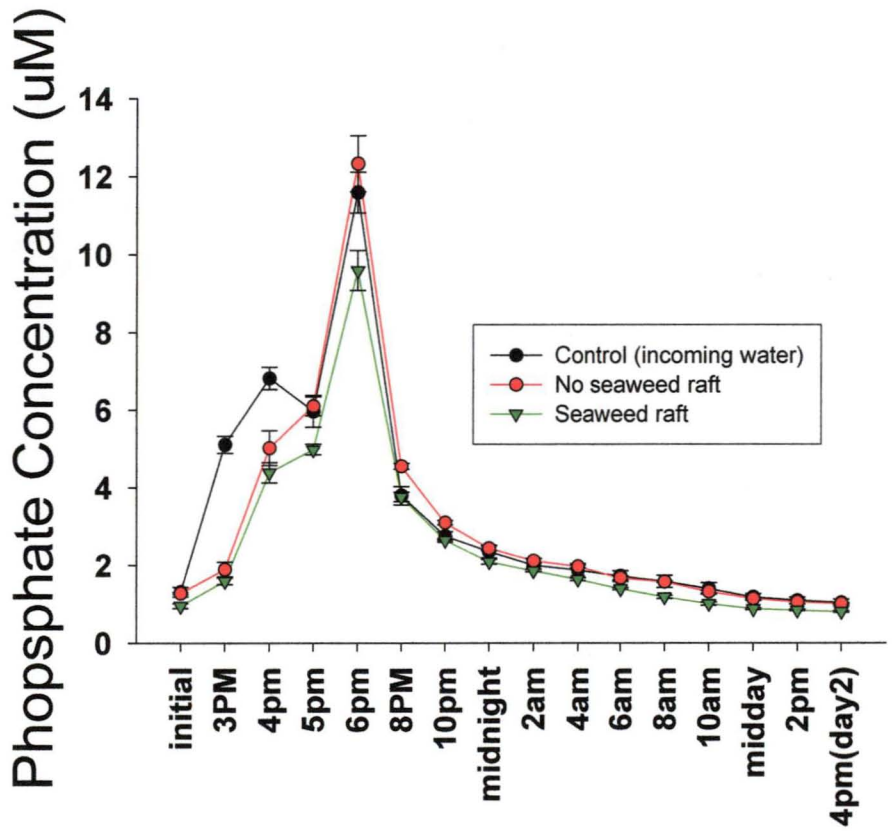

Figure 5.4: Phosphate concentration for system inflow control water, effluent tank water without seaweed raft and with seaweed raft (control $n = 3$, treatments $n = 4$ mean ± SE)

Nitrate concentrations showed statistically ($P < 0.5$) lower concentrations in the seaweed raft treatment than the no seaweed raft treatment (Fig. 5.5). On a number of

occasions throughout the experiment the no seaweed raft treatment showed significantly greater concentrations than the inflow water indicating some production of nitrate.

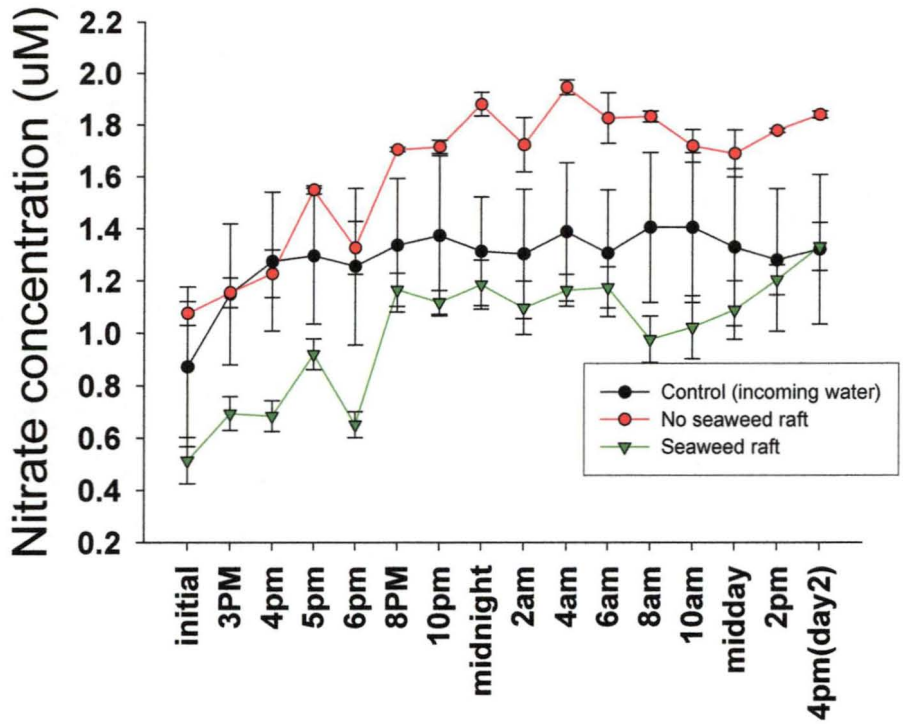

Figure 5.5: Nitrate concentration for system inflow control water, effluent tank water without seaweed raft and with seaweed raft (control $n = 3$, treatments $n = 4$ mean ± SE)

Nitrite concentrations for each of the treatments showed similar patterns as exhibited by nitrate, with the no raft treatment exhibiting greater nitrite levels than the system inflow concentrations (Fig. 5.6), and statistically lower concentrations than the raft treatments ($P < 0.05$). Unlike the nitrate treatment however there was a decrease in concentration of all treatments at 6pm followed by a sharp rise.

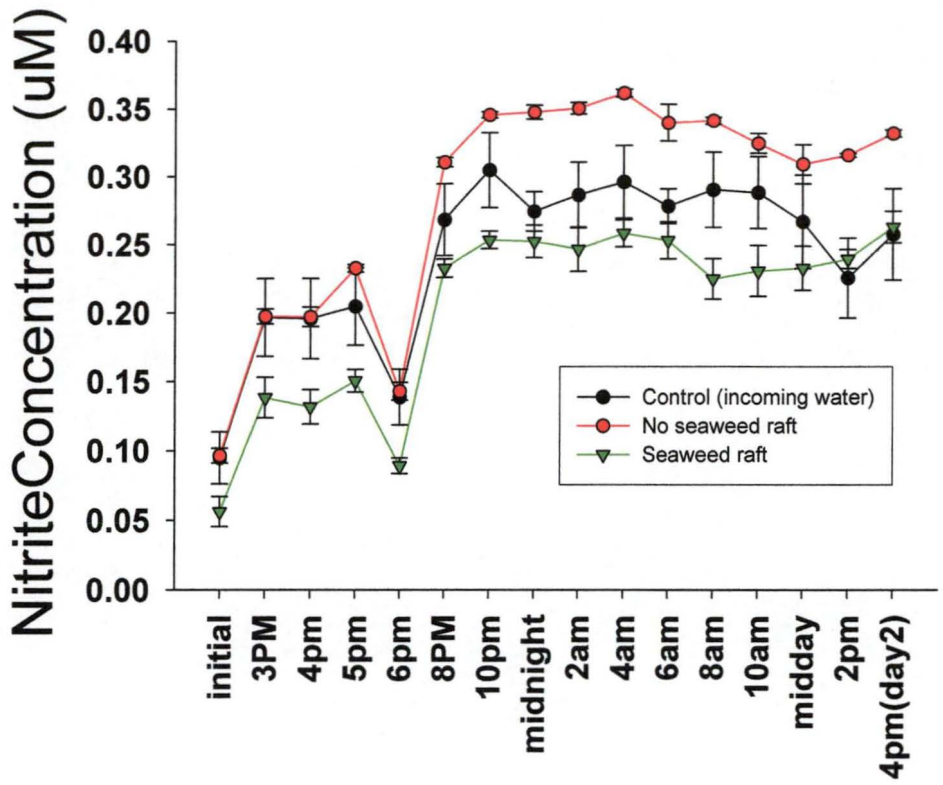

Figure 5.6: Nitrite concentration for system inflow control water, effluent tank water without seaweed raft and with seaweed raft (control $n = 3$, treatments $n = 4$ mean ± SE).

Light intensities ranged from between 1.5 –0.58 x 10^{17} quanta/second/cm^2 (Fig. 5.7). Initially light intensity decreased as the first afternoon progressed and by 8:30pm there was no detectable light. Irradiance remained at zero until 7am when it began to increase before a peak at 8:45am.

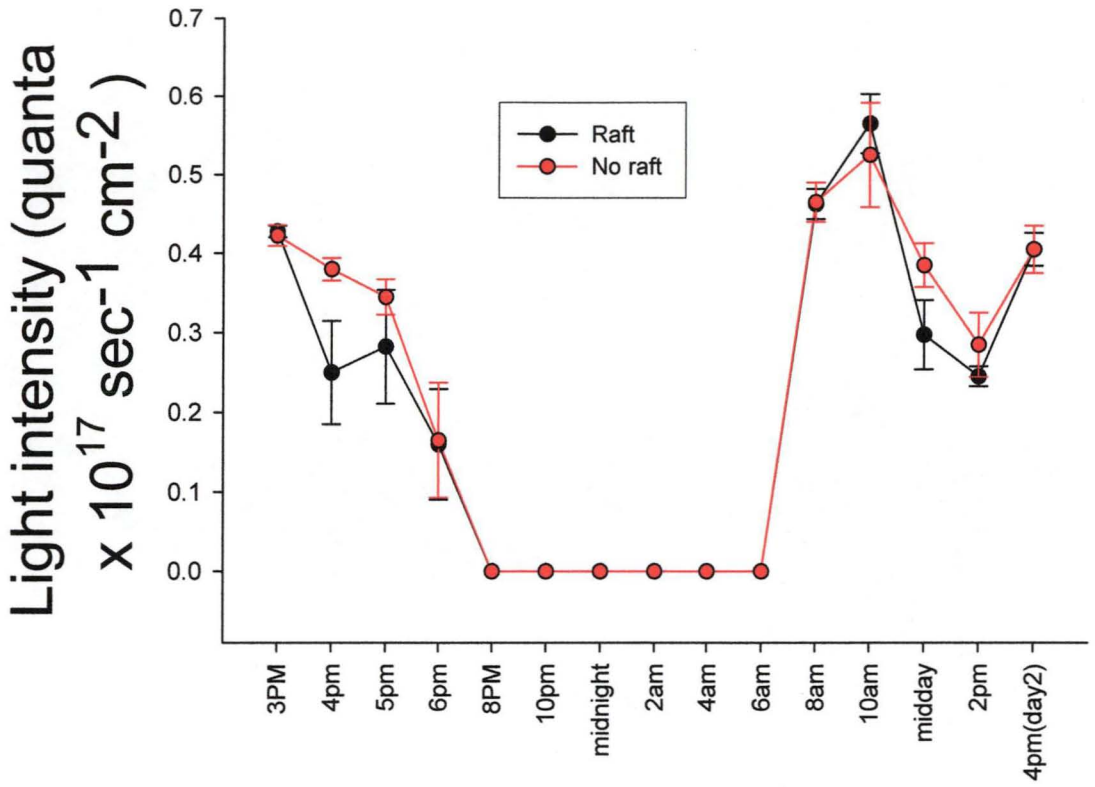

Figure 5.7: Mean light intensity for seaweed raft and no seaweed raft treatments ($n = 4$, mean ± SE).

Temperature in the raft treatments remained lower than the no seaweed raft treatment during the late afternoon to early morning hours while the inverse was true during the daytime hours (Fig. 5.8). Over the course of the 24 hour period there was approximately a 5 degree variation in temperature with the greatest rate of change occurring around midday (increase) and 5pm (decrease).

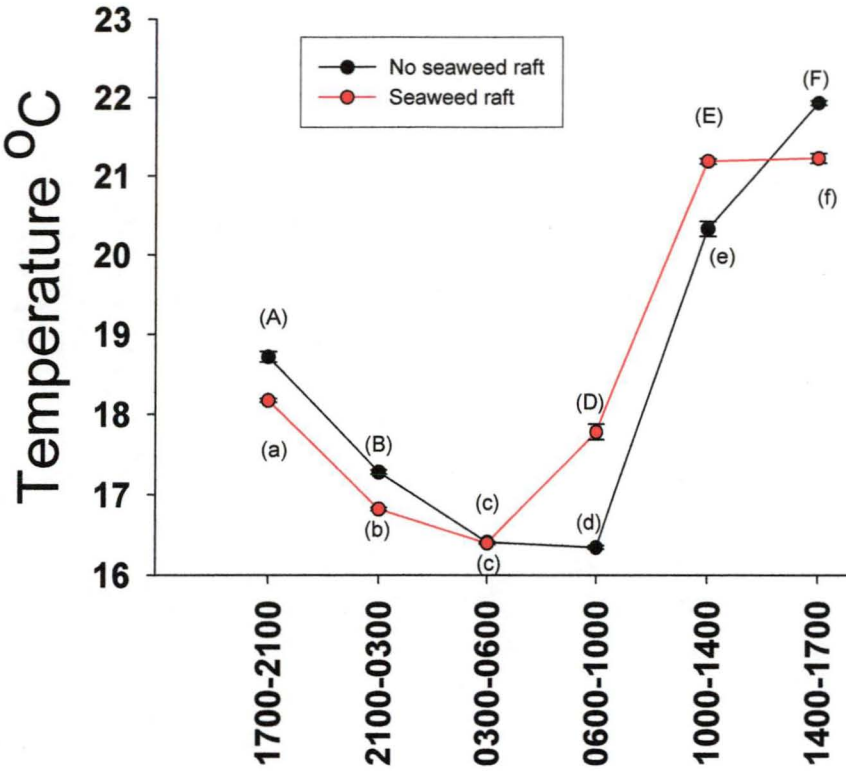

Figure 5.8: Temperature profiles for tanks with seaweed rafts and without seaweed rafts over 24 hours. All values within each time are significantly different from each other (i.e. (A) and (a) are significantly different)

Dissolved oxygen concentrations followed a similar pattern to temperature with lower oxygen concentrations during the late afternoon to night time hours and greater concentrations during the daytime hours (Fig. 5.9).

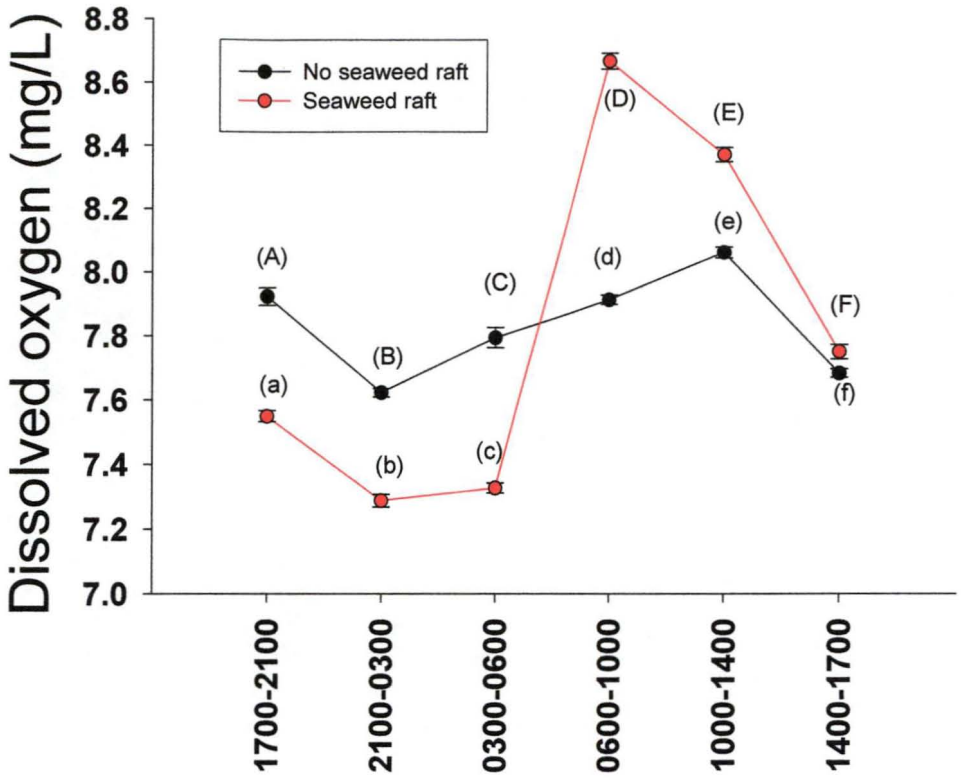

Figure 5.9: Temporal dynamics of dissolved oxygen concentrations in tanks with seaweed rafts and without seaweed rafts over 24 hours. All values within each time are significantly different from each other (i.e. (A) and (a) are significantly different)

Turbidity was slightly greater in the seaweed raft treatment than the no seaweed raft treatment between 5-9pm ($P < 0.05$), however remained lower than the no seaweed raft treatment for the majority of the sampling periods (Fig. 5.10).

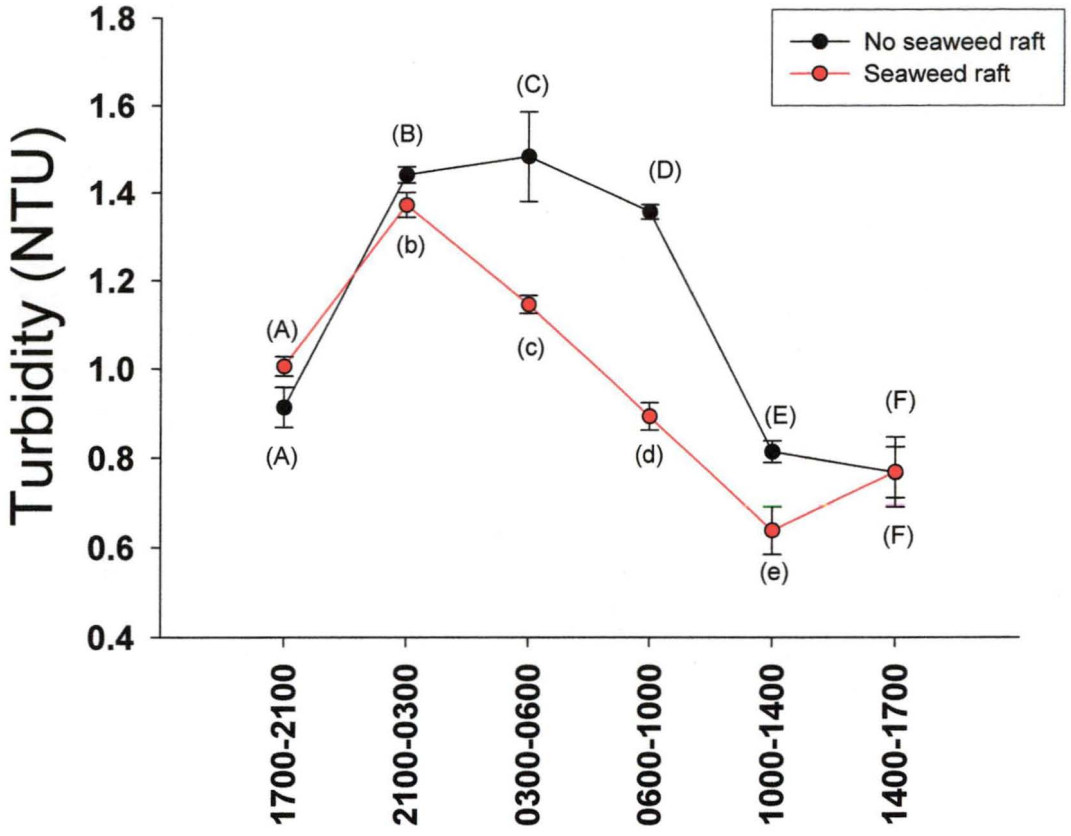

Figure 5.10: Temporal dynamics of turbidity in tanks with seaweed rafts and without seaweed rafts over 24 hours. All values within each time are significantly different from each other (i.e. (A) and (a) are significantly different)

5.4 Discussion

The results of the experiment indicate that the seaweed rafts were capable of reducing effluent nutrients, particularly inorganic nitrogenous dissolved waste; and also altering other water quality parameters such as temperature and turbidity.

Ammonium is taken up by marine algae preferentially over other nitrogen species as it can be directly incorporated into amino acids. This is in contrast to nitrite and nitrate

both of which require reduction before assimilation (Lobban and Harrison, 1994). From figure 5.2 it can clearly be seen that the seaweed rafts have the effect of reducing ammonium concentration of the effluent waters by up to 71%. On average however if we calculate daily loads from the flow rates of the experimental tanks multiplied by the concentration, the average overall daily ammonium export load would decrease by 48%. This represents a significant reduction in ammonium and hence indicates that at least under the given conditions of the experiment that the effluent is likely to be capable of supplying seaweeds with some nutrients for their maintenance and growth. While this rate of uptake may not be indicative of the year round capacity of the rafts to absorb ammonium (Campbell, 1999; Phillips and Hurd, 2000) (extrapolation to the commercial situation and to annual performance is discussed later), it demonstrates that seaweed attached to rafts can be moderately efficient in reducing effluent nutrients, in some cases reducing much greater than unattached seaweed studies, and in other cases not quite achieving the same level of reduction.

That silicate concentrations were not significantly different between the seaweed and no seaweed raft treatments indicates that silicate is neither being produced or consumed by the seaweed. Production of silicate in the fibreglass experimental tanks was unlikely due to the limited sources of silicate, however the utilisation of silicate by algae (i.e. epiphytic diatoms on the macrophytes) was expected. Commonly diatoms are a scavenging algae species within abalone culture systems (Piehler et al., 2004) however there may be a number of reasons why such communities had not established. They include the shading effect of the rafts, the relative 'immaturity' of the tanks with respect

to community establishment and also the shorter residence times of the tanks (i.e. flushing of algal biomass).

The consistently lower average concentrations of dissolved inorganic P throughout all sampling periods in the seaweed relative to the no seaweed raft treatments indicates that there may be some degree of P utilisation by the seaweeds. While a clear difference between treatments is shown for ammonium, seaweed P requirements are 16 times lower than N (Lobban and Harrison, 1994) which is consistent with the smaller amount of uptake.

The no seaweed raft treatment contained greater concentrations of both nitrate and nitrite than the seaweed raft treatment indicating the possible production of nitrate and nitrite rather than consumption by the seaweed rafts (i.e. due to preferential uptake of ammonium). Conditions were suitable for nitrification (Ling and Chen, 2005; Sharma and Ahlert, 1977) as there are high concentrations of ammonium available, the system inflow water was likely relatively low in organics (i.e. is drawn from high in the water column near the tank outflow), and there was a great deal of aeration and hence mixing. Interestingly enough there was also depressed concentrations for both nitrate and nitrite at around 6pm when compared to other sampling times. This dip in concentration may be related to feeding times and perhaps an elevated concentration of organics given that heterotrophic bacteria tend to outcompete nitrifying bacteria under such conditions (Hargreaves, 1998).

The rafts caused lower dissolved oxygen concentrations during the late afternoon and night time between approximately 5pm and 6am. This is typical of dissolved oxygen concentrations in aquaculture systems where primary production is a dominant process

(Brune et al., 2003; Sumagaysay-Chavoso et al., 2004). Commonly during the day photosynthetic activity produces oxygen, causing elevated dissolved oxygen concentrations, while at night the seaweed respire causing lower dissolved oxygen concentrations. Given that abalone are nocturnal, feeding activity and movement place demands on dissolved oxygen concentrations and the seaweed rafts further compound these demands. This may or not represent a problem depending upon the farming situation, however within the experimental system there was a maximum deficit of 0.5 mg/L during the night between the raft and no raft treatments. There was also a maximum 0.8mg/L positive difference with rafts during the day. In the commercial production tanks with the appropriate management of aeration flow rates these DO variations may be reduced or eliminated. However while the trends exhibited are likely to be similar between the experimental system and AFA tanks, the absolute amounts of oxygen deficit and surplus are unlikely to be any more extreme as the experimental system had a 2 fold greater surface area of raft per litre of water indicating that there is likely to be a greater oxygen demand in the experimental system than in the standard AFA grow-out tank conditions.

For the majority of the day turbidity was lower in the seaweed raft treatment than the no seaweed raft treatment. However, around the time the formulated feed entered the water turbidity became similar in both treatments with little or no difference between them. One possible fate of the particulates in the seaweed raft treatments is likely to be adsorption on the surface of the seaweed thallus. It should be noted that while the seaweed rafts may be reducing particulates, there is clearly a limited capacity of the

seaweeds to adsorb particulates before they become fouled and restricted in gas and nutrient exchange.

Over the 24hour experimental period, similar temporal trends were exhibited between the concentrations of nutrients in the inflow water (to the experimental system) for both the raft and no raft treatments. This indicates that the predominant trends observed in all treatments are driven by the diurnal changes in the grow-out tank effluent concentrations. The results of this experiment indicate that there are a suite of conditions under which the rafts have the ability to reduce effluent nutrients. To apply experimental results to a commercial scale we need to consider two key factors. Firstly the differences between the experimental system and the real grow-out tank systems, and also the annual changes in environmental conditions.

The main difference between the experimental system and the AFA grow-out system that is likely to affect nutrient uptake is the longer residence times of the AFA grow-out tanks versus the experimental system. The residence times of the experimental tank were approximately 3-4 times shorter than the standard AFA grow-out tanks and hence it is highly likely that if the rafts are placed into standard AFA grow-out tanks that they may have a greater effect on nutrient reduction.

Another clear difference between the experimental system and the AFA grow-out tanks is that the experimental system employed fibreglass tanks, while AFA grow-out tanks are cement giving a much greater surface area for colonisation by microalgae and bacteria. This difference is likely to have a limited effect on the performance of the seaweed rafts. However the shading effect of the rafts is likely to cause lower levels of

light to penetrate onto the walls of the tank and potentially reduce internal tank consumption of nutrients.

The second factor that might limit extrapolation of the experimental tanks results to the grow-out tanks may be the temporal variation in environmental conditions experienced through the year. The present study was conducted in summer under favourable seasonal conditions for seaweeds and is likely to represent a 'best case' scenario for nutrient uptake by seaweeds. Late summer under conditions of good water movement (for gas and nutrient exchange), warm water temperatures (faster processes), adequate supply of nutrients, long daylength (for maximum photosynthesis) and good water exchange were recorded. It is therefore likely that these conditions may represent closer to optimum conditions for the seaweed raft, relative to the conditions that are likely to be experienced over winter. During winter environmental factors governing nutrient uptake in seaweeds, may be sub optimal causing limitation of the remediation capacity and lower growth of the seaweeds on the rafts.

The results of this experiment however show that seaweed attached to PVC plastic and cultured within the same system as the abalone are able to reduce effluent nitrogen loads by approximately 48%. There is minimal effect of the seaweed rafts on other water quality parameters and if the rafts are can be maintained in a low cost manner appear to provide abalone with shelter, food source and improved water quality.

CHAPTER 6: Tasmanian abalone farms environmental monitoring program

6.1 Introduction

Even a full environmental study of the environmental impacts of a single farm such as, Abalone Farms Australia (AFA), is of limited use to decision makers applying regulation across a spectrum of farms. Legislators need tools and information for policies that seek to manage the entire abalone aquaculture industry. Therefore while comprehensive environmental information for a single farm is available, depth of research within the abalone industry is required before any information found in one study of a single farm is can be seen to be representative of an industry.

While it may be ideal to do complete environmental impact studies of each and every abalone farm, this is an impractical and economically unviable approach. A more cost efficient approach may be to have less intensive environmental monitoring on farms, and use the results of more intensive studies (i.e. AFA) to determine the likely impact of other farms on the marine environment. Currently there are a number of abalone farm effluent monitoring programs around Australia. While the fundamental aim of these monitoring programs is to ensure that environmental integrity is maintained in the marine environment, these monitoring programs have limited value without the background knowledge of the causes of these results.

Potentially there are many variables that may cause problems in relating the results of one abalone farm's environmental performance (i.e. net export of nutrients), to the environmental performance of another farm. Some of the possible variables include:

1. Abalone biomass
2. Solid Separation Device presence/absence and efficiency
3. Seasonal variations in daylength, rainfall, water temperature
4. Sedimentation pond conditions (i.e. biota and sediment quantity and quality)
5. Farm design – including tank design, drain lengths, sedimentation pond residence time
6. Ambient water quality - particularly temperature, dissolved oxygen, salinity and primary production (Chl a)
7. Health of abalone within farm
8. Farm residence times
9. Abalone diet used
10. Species of animal cultured
11. Husbandry variables – feeding strategies, cleaning frequency and stocking densities, aeration and water movement within tanks.

The nature of the receiving environment for the effluent waters also is another factor that is likely to cause much variation in the actual biological environmental impact upon the marine environment.

1. Coastline weather conditions - energy/wave exposure, wind direction, currents
2. Farm outflow position i.e. subtidal/intertidal/above high tide level
3. Receiving environment substrate type (i.e. sandy beach versus rocky shore)
4. Receiving environment bathymetry
5. Nearshore intertidal and subtidal marine community structure

Some of these factors may be relatively simply accounted for (such as temperature range), while other variables such as husbandry are more qualitative and difficult to account for. The largest factors determining effluent quality for AFA appears to be the formulated feed input and abalone biomass of the farm (Chapter 2a). These two factors are not independent as clearly the abalone biomass is a significant factor determining the amount of formulated feed input into the culture system. Given that biomass and feed rates are important for good animal husbandry and economic management of aquaculture operations, they are commonly monitored on farms around Australia. Therefore a model that predicts effluent nutrient concentrations based upon one or more of these parameters may be a useful tool for environmental management of farms with similar farming systems.

Within Tasmania the majority of farms operate using cement deepwater culture tanks with long residence times. While the exact dimensions and shapes may differ between farms, the differences remain relatively small compared with many South Australian farms which use shallow high flow 'slab' systems or PVC pipes. Subsequently given the relative uniformity of culture systems within Tasmania, it may be possible to extrapolate nutrient loads based upon the data for AFA. One point of difference which is likely to cause great variation between different farms in Tasmania is the presence and absence of Solid Separation Devices (SSD). Studies have shown that the process of remineralisation from aquaculture waste sediments is likely to play a large role in the nutrient dynamics of an operation (Burford and Lorenzen, 2004; Hargreaves, 1997) by increasing N and P water column concentrations. Subsequently farms with SSDs are

likely to exhibit different nutrient dynamics to farms without SSDs. In addition to the presence and absence of SSDs, there is also the efficiency of the SSDs to retain particulates. The sum of these differences culminates in a difference of effluent nutrient composition (particulate and dissolved) and hence environmental impact as particulates and dissolved nutrients impact the environment in different ways (Crawford, 2004).

This chapter examines the variability in nutrient loads exported for different farms within the Tasmanian abalone industry and the driving forces of variability which may exist between these farms.

6.2 Materials and Methods

Two to three monthly water quality samples were taken from the farm inflow and farm outflow waters of three commercial abalone farms (infrastructure to produce 20 tonnes of abalone annually) around Tasmania for over a period of approximately 12 months. Samples were taken and analysed in triplicate for Total Suspended Solids (TSS) and dissolved nutrients (ammonium, nitrate, nitrite, phosphate and silicate) according to the methods outlined in Chapter 3. In addition to the above water quality sampling, information about the farm operations such as: feed rates, biomass, water flow rates, sedimentation pond size, residence times and diet used were all noted.

6.2.1 The farms

The three farms were chosen for monitoring based upon their production capacity, willingness to be involved and presence/absence of a sedimentation pond. One farm was based in the south of Tasmania (Farm A), one on the east coast of Tasmania

(Farm B) and one in the north of the state (Farm C). Each of the farms had infrastructure available to produce a minimum of 20 tonnes of abalone per annum, but all were at varying stages of development. Farm A had been in operation for approximately 8 years and was at the stage where abalone were consistently harvested from the farm and biomass was relatively stable but increasing over the period of sampling. Farm B had been established for over 20 years, was harvesting animals and had a total farm biomass which was reported by the farm operators to have a minimal yet undefined variation over the period sampled. However only an extremely approximate biomass and feed rate information for Farm B was obtained and hence limited results for this establishment are presented here. Farm C on the other hand was relatively new and hence not at the stage where abalone were being sold to markets. Over the monitoring period biomass was always increasing.

Farm statistics were taken for all farms and are presented in Table 6.1. All farms employed similar farming styles using cement walled deep water tanks (commonly approximately 6m(length)x 2m(width)x 0.4m (deep)) with ambient seawater flowing through and aeration. While all the farms monitored used Adam and Amos formulated feeds, none of the farms used the same type of diet used at AFA. Two of the three additional farms had sedimentation ponds; Farm A had a cement lined pond, Farm C had an earthen pond and Farm B had no sedimentation pond. AFA had a PVC lined sedimentation pond.

Table 6.1: Miscellaneous farm statistics for three abalone farms and AFA monitored over the duration of 12 months around Tasmania. Note: figures are approximation

	FARM A	FARM B	FARM C	AFA
Biomass (tonnes)	17-30	50	4-8	17-20
Feed manufacturer	Adam and Amos	Adam and Amos	Adam and Amos	Adam and Amos
Predominant diet type	5mm chip	3 and 5mm chip	3mm chip	1mm noodle
Farm water flow rates (ML/day)	11.3	27.7	15.9	8.6
Sedimentation pond volume (L)	21,750	No sedimentation pond	2,374,000	1,500,000
Sedimentation pond substrate	Cement	No sedimentation pond	Earthen	Plastic lined
Discharge environment	Shallow coastal bay	Sandy beach	River mouth –estuarine environment	Coastal
Tank type	Cement deep water	Cement deep water	Cement deep water	Cement deep water

6.3 Results

6.3.1 Monitoring program

Farm A and Farm B consistently exported dissolved nitrogen ($NH_4^+ + NO_x$), phosphate and silicate (Farm B showed one incidence of consumption of silicate) over the periods sampled, while Farm C which showed variable results with both the export and consumption of nutrients occurring on some occasions (Figs. 6.1, 6.2, 6.3).

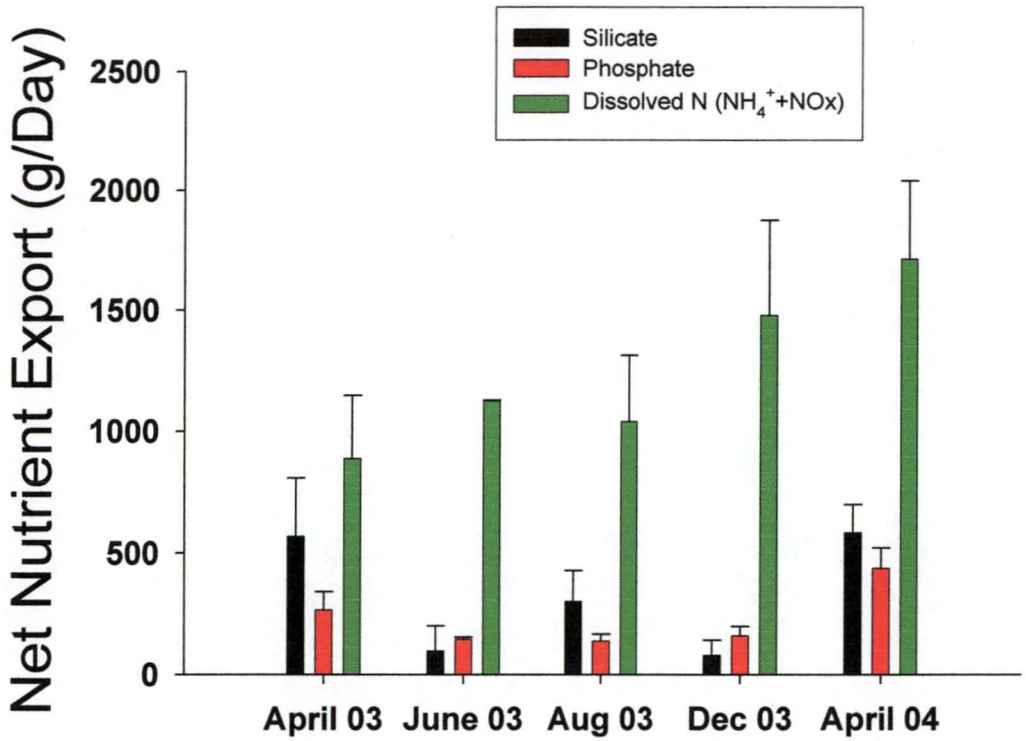

Figure 6.1: Nutrient export (g/day) results for the environmental monitoring of Farm A. Values given are the result of mean farm outflow – mean farm inflow nutrient concentration (n=3) multiplied by farm water flow rate.

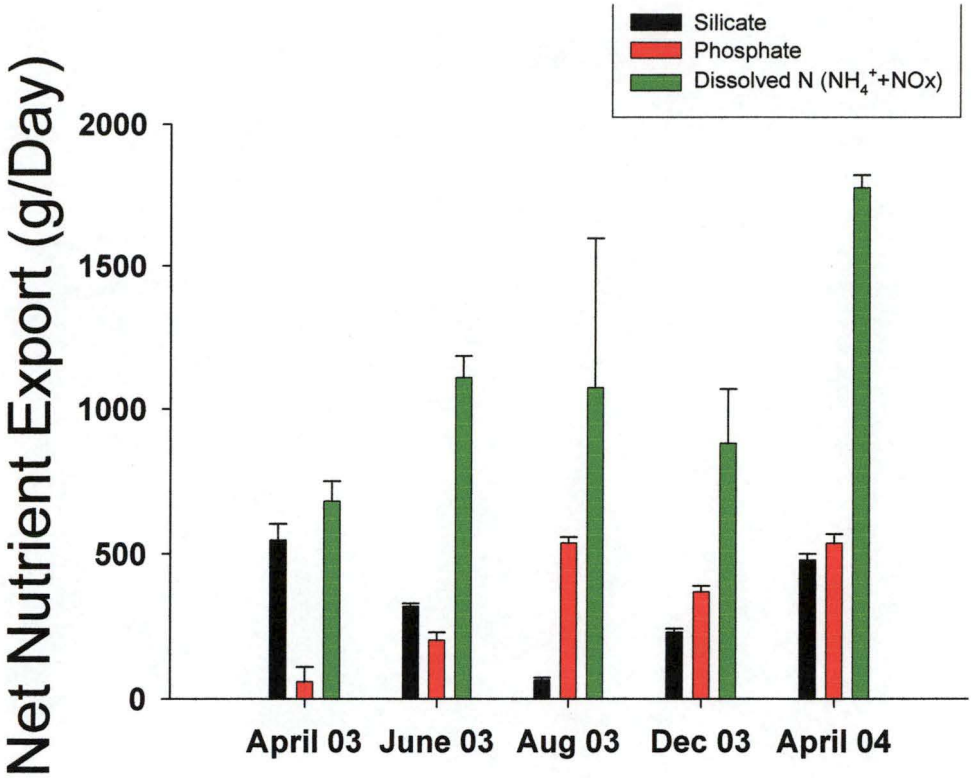

Figure 6.2: Nutrient export (g/day) results for the environmental monitoring of Farm B. Values given are the result of mean farm outflow – mean farm inflow nutrient concentration (n=3) multiplied by farm water flow rate.

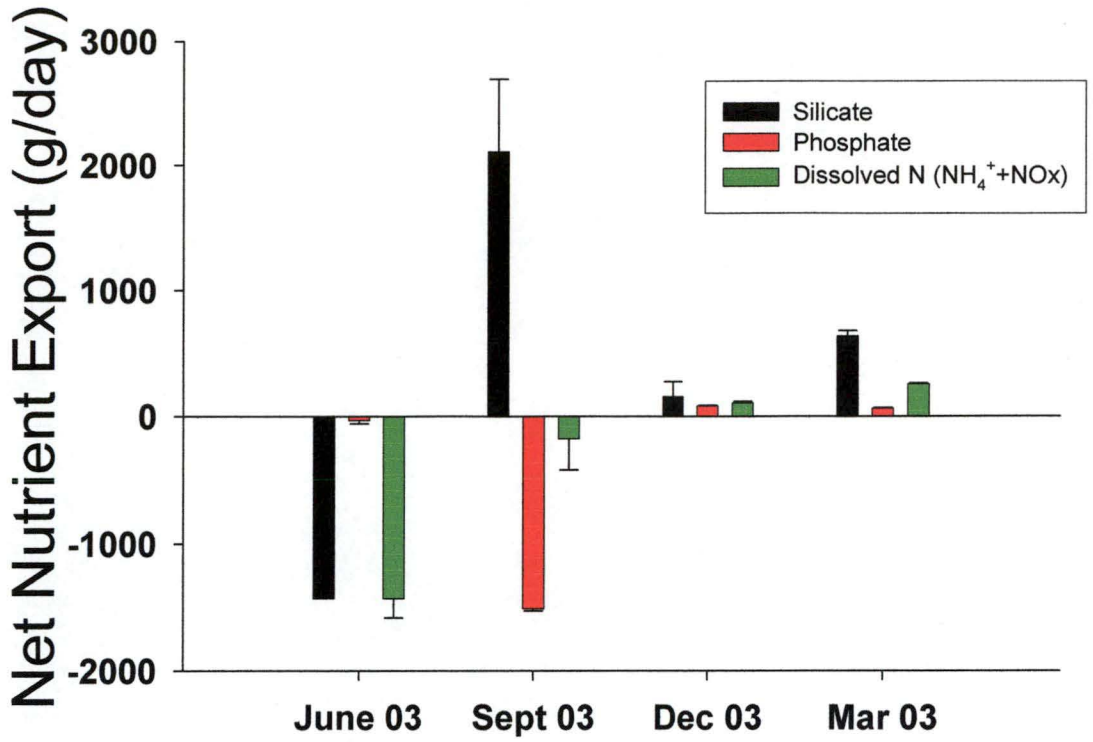

Figure 6.3: Nutrient export (g/day) results for the environmental monitoring of Farm C. Values given are the result of mean farm outflow – mean farm inflow nutrient concentration (n=3) multiplied by farm water flow rate.

Farm C predominantly showed a consumption of nutrients during the first half of the monitoring program, while the second two sampling periods showed production of relatively low levels dissolved nitrogen (consumption of 308g/day), silicate (965g/day) and phosphate consumption of 349g/day). Dissolved nitrogen was the greatest of all nutrients exported amongst Farms A + B with up to two kilograms of dissolved N being exported daily, while silicate and phosphate loads averaged 325 and 228 g/day respectively for Farm A, and 193 and 347 g/day respectively for Farm B.

Mean nutrients over the sampling period showed similar loads of silicate (range = 190-300g/day), phosphate (range = 170-340g/day) and dissolved nitrogen (range = 700-

1250g/day) for AFA, and Farms A and B; however Farm C showed high production of silicate (965g/day) and consumption of phosphate (-349g/day) and dissolved nitrogen (-308g/day) (Fig. 6.4).

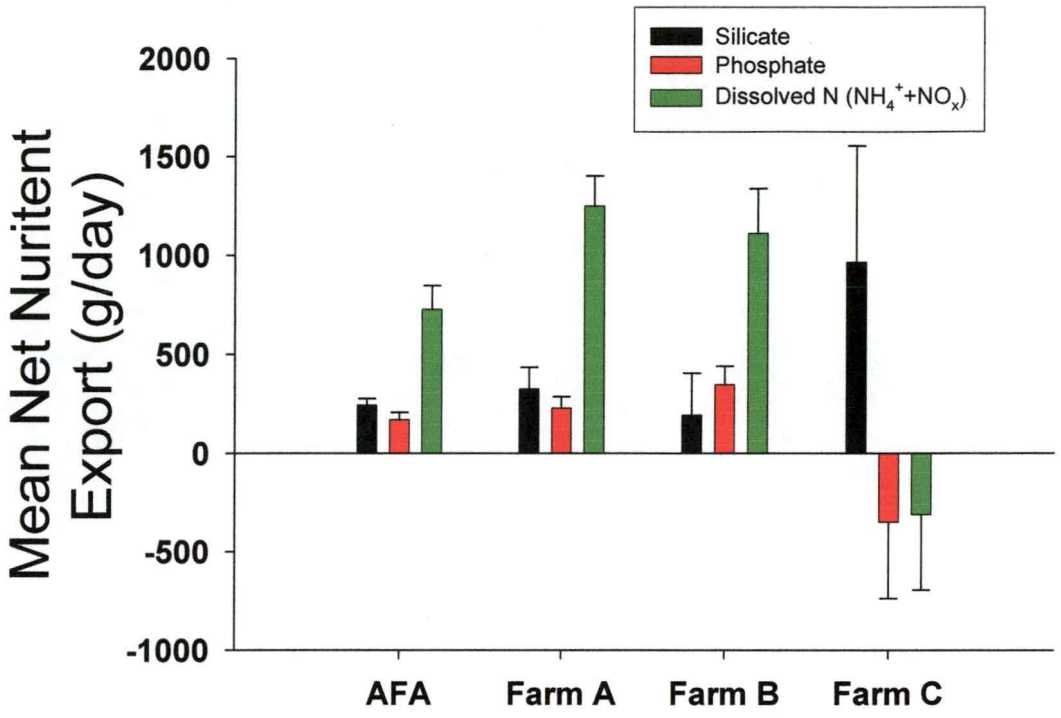

Figure 6.4: Mean nutrient export (g/day) results for the environmental monitoring of all farms over the 12 month period to April 04. Values (mean ± SE) given are the result of mean of mean farm outflow – mean farm inflow nutrient concentration multiplied by farm water flow rate (n=11, 5, 5 and 4 for AFA, and Farms A,B,C respectively).

Farm A showed greater mean suspended solids concentrations at the farm outflow compared with the farm inflow (Fig. 6.5) with a statistically significant difference between the farm inflow and farm outflow (t = -3.25, P=0.047).

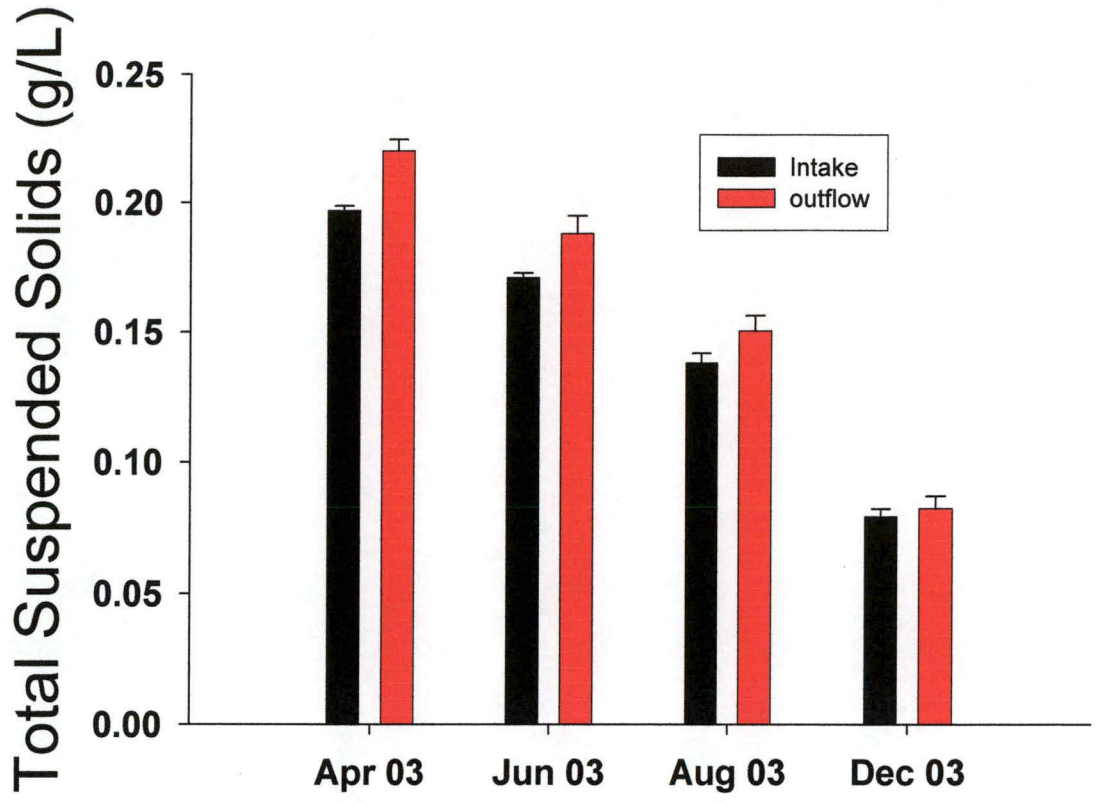

Figure 6.5: Total Suspended Solids (g/L) for the environmental monitoring of Farm A at the farm inflow and farm outflow.

Farm B showed significantly greater mean concentrations of suspended solids at the outflow (mean difference = +0.047 g/L) indicating a net export of particulates (t = -3.36, *P*=0.044) (Fig 6.6) than the farm inflow.

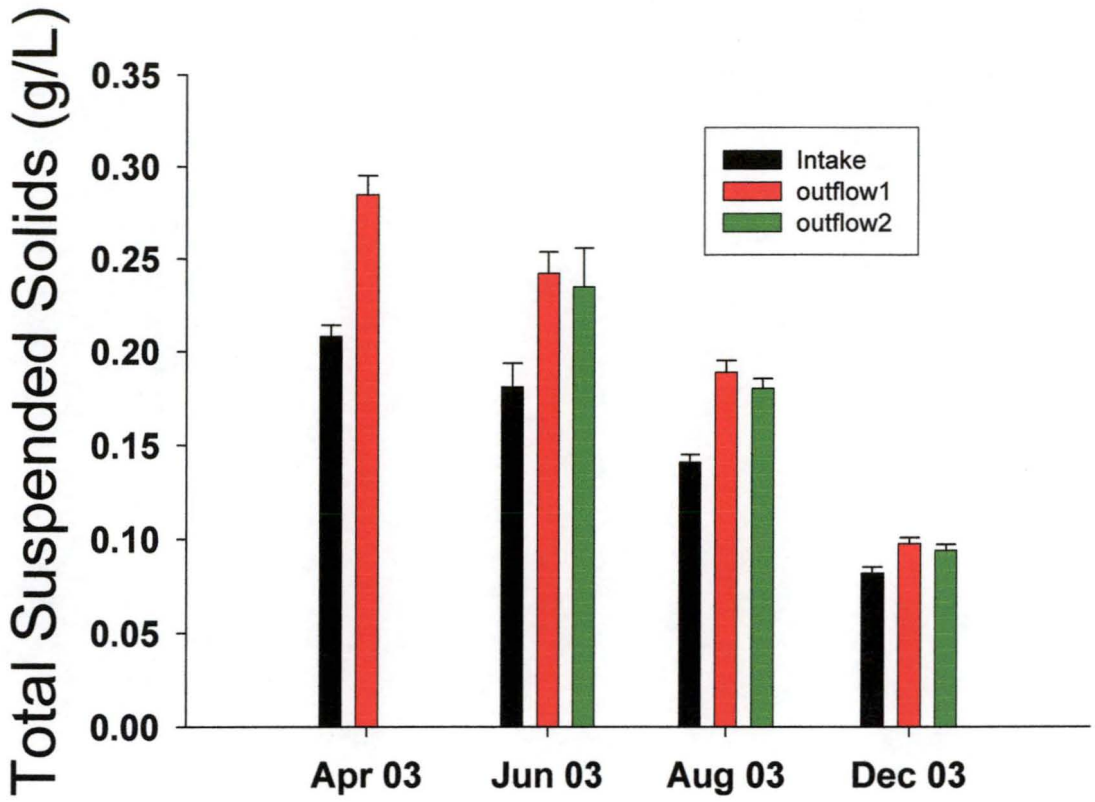

Figure 6.6: Total Suspended Solids (g/L) for the environmental monitoring of Farm B at the farm inflow and farm outflow.

Farm C showed no significant difference between the mean farm inflow and farm outflow TSS concentrations ($t = 0.87$, $P=0.47$) (Fig. 6.7) (mean difference = - 0.00057 g/L).

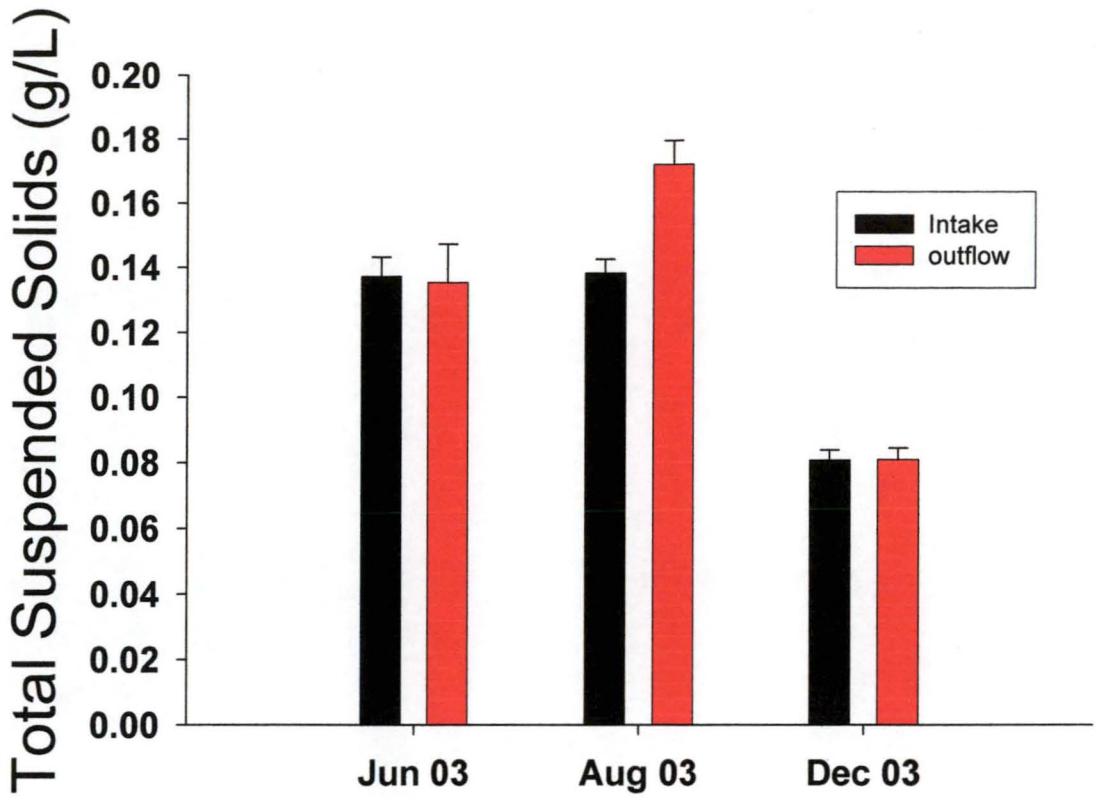

Figure 6.7: Total Suspended Solids (g/L) for the environmental monitoring of Farm C at the farm inflow and farm outflow.

Figure 6.8 shows the mean farm inflow and farm outflow values for all samples taken at AFA and Farms A, B and C. AFA and Farm C both show that there is very little difference between the mean sampling, while Farms B and A show evidence of greater average TSS concentrations at the farm outflow compared to the farm inflow.

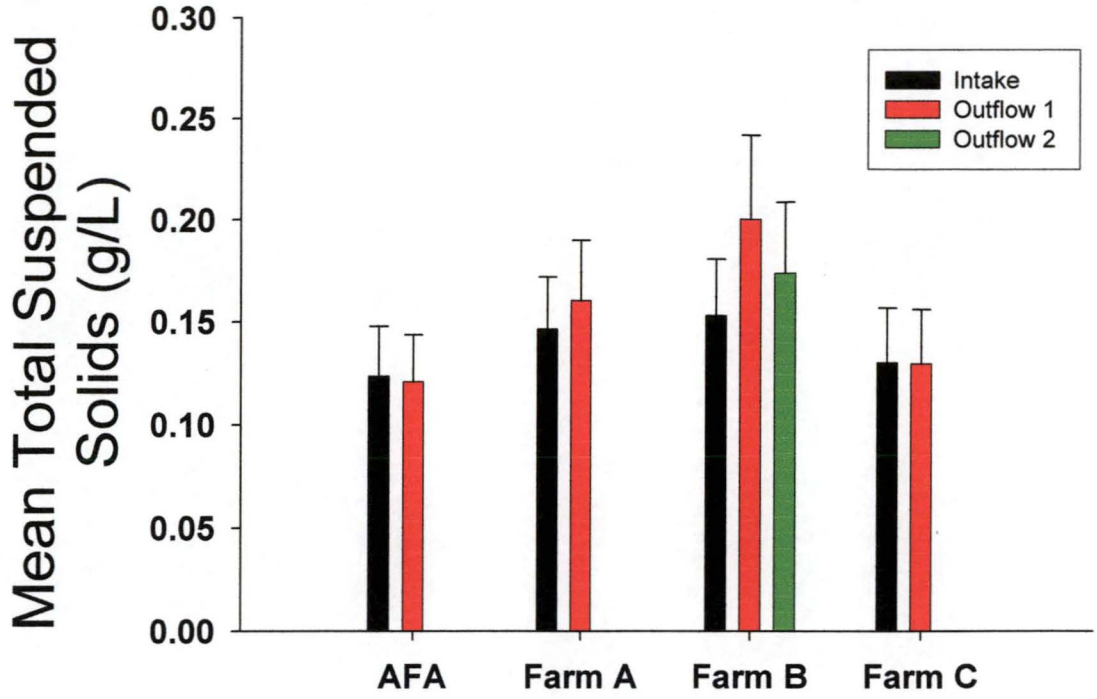

Figure 6.8: Mean TSS for farm inflow and farm outflow (g/day) for the environmental monitoring of all farms over the 12 month period to April 04. Values (mean ± SE)

Daily feed rate and dissolved nitrogen data for farms A and C (farm B not added as only very approximate feed data was available) were added to the feed versus nutrient relationship found for Abalone Farms Australia (Chapter 3). The resulting data set showed a significant linear relationship between the dissolved nitrogen and daily feed rate (Fig. 6.9) ($P = 0.001$, $n = 15$, $r^2 = 0.708$).

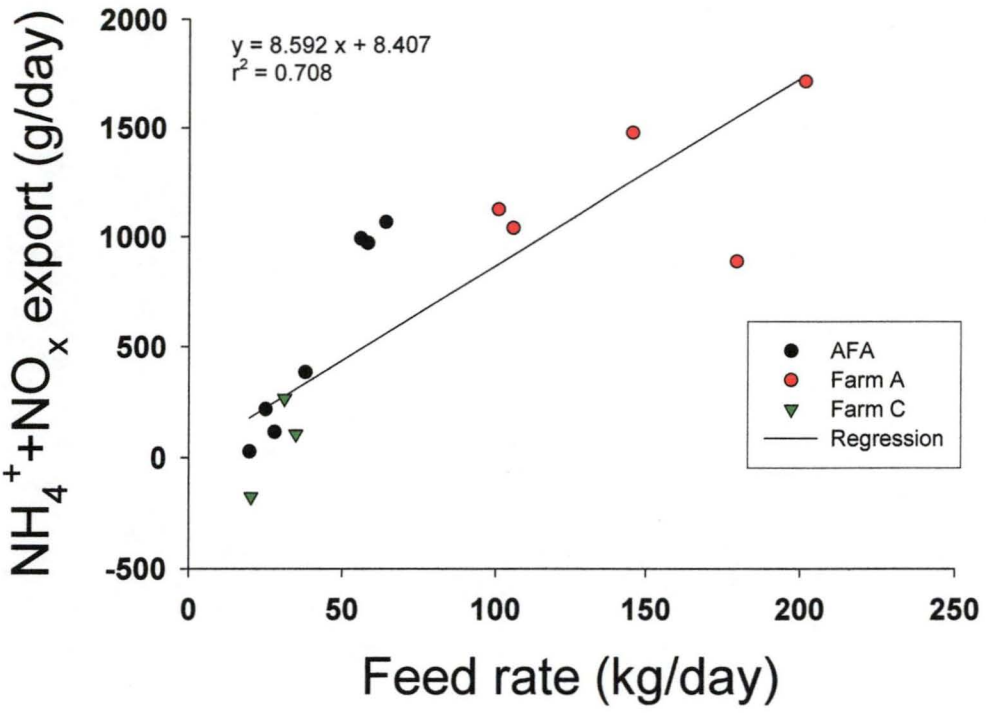

Figure 6.9: Regression of dissolved $NH_4^+ + NO_x$ exported versus daily feed rate for three abalone farms around Tasmania.

A similar relationship was also found for the farms' biomass of abalone and dissolved nitrogen export with (Fig. 6.10) ($P<0.001$, $n= 12$ $r^2 = 0.851$).

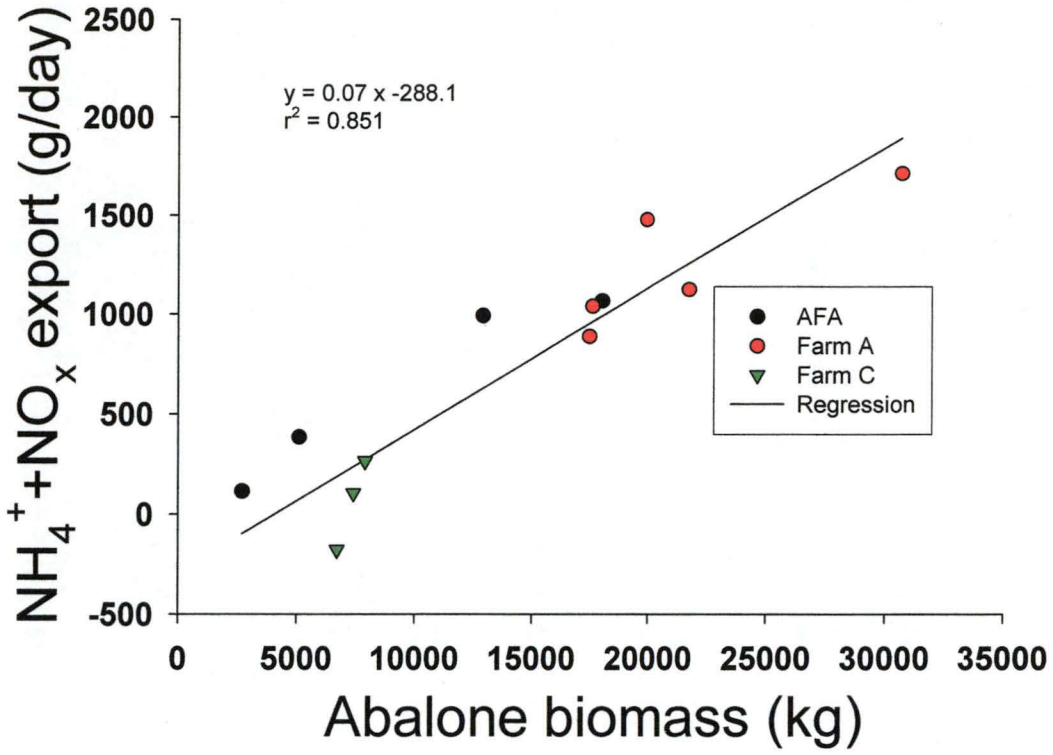

Figure 6.10: Regression of dissolved $NH_4^+ + NO_x$ exported versus abalone biomass for three abalone farms around Tasmania.

Daily P export showed a significant linear regression of daily feed rate (Fig. 6.11) (P=0.012, n=11, r^2=0.621) and a significant correlation with the farms' biomass of abalone (Fig. 6.12) (P= 0.002, n=11, r^2=0.665).

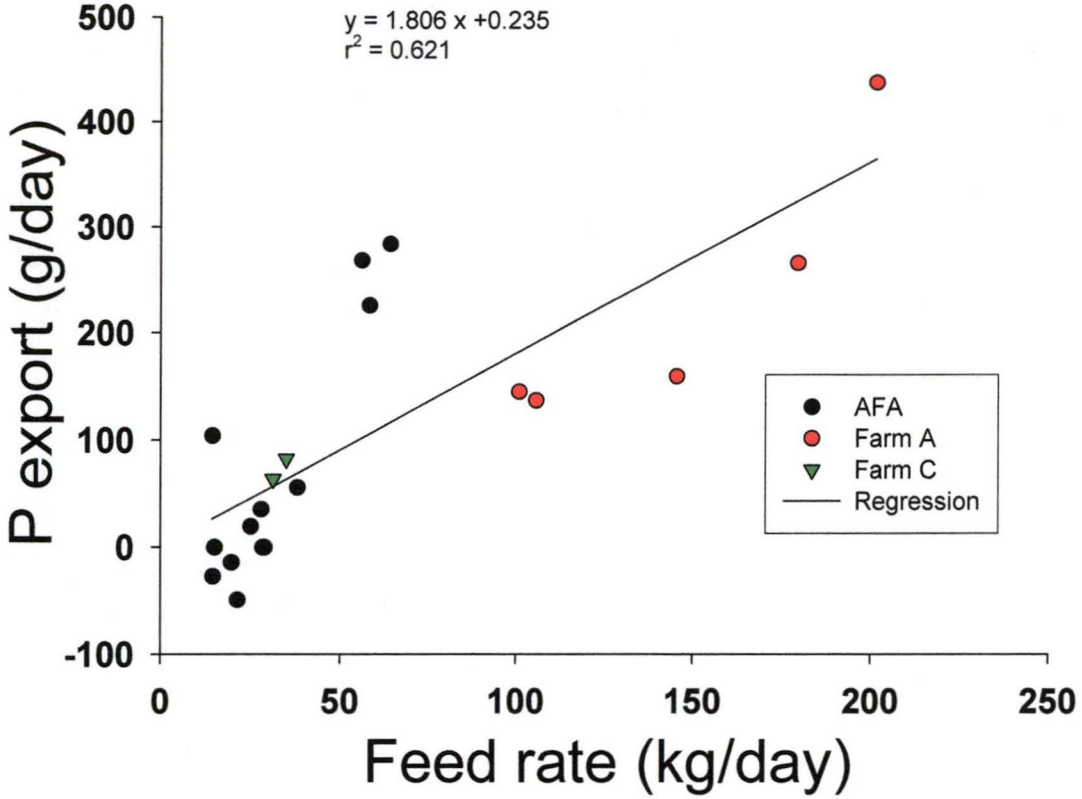

Figure 6.11: Regression of dissolved P exported versus daily feed rate for three abalone farms around Tasmania.

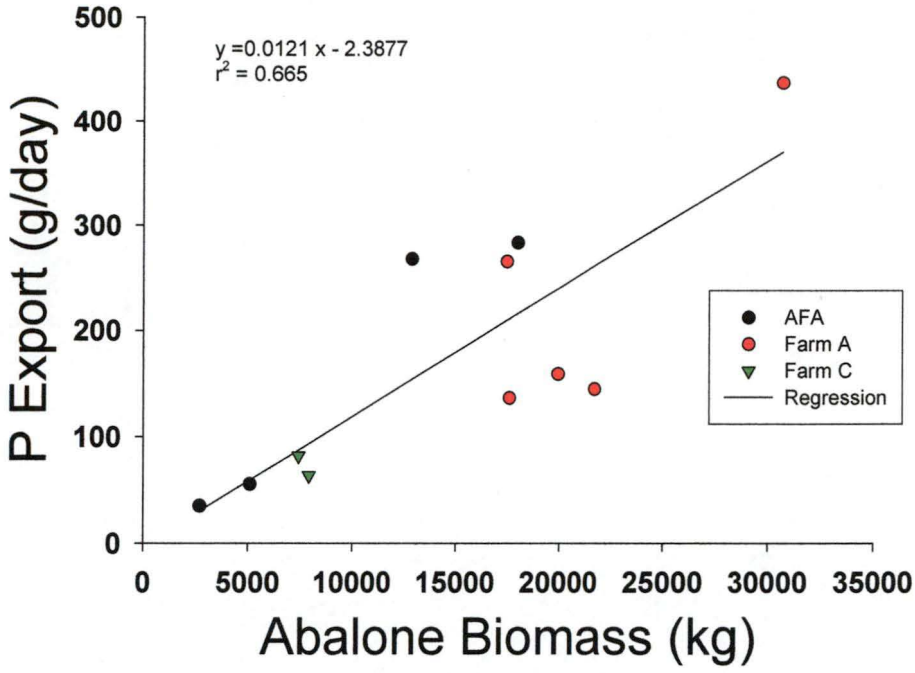

Figure 6.12: Regression of dissolved P exported versus abalone biomass for three abalone farms around Tasmania.

Figure 6.13 shows a relationship between sedimentation pond residence times and the daily amount of TSS export. AFA showed the lowest amount of particulates exiting the farm outflow, and Farms A and C with shorter sedimentation pond residence times tended to have slightly greater TSS export, while Farm B, which had no sedimentation pond, produced a greater amount of suspended solids.

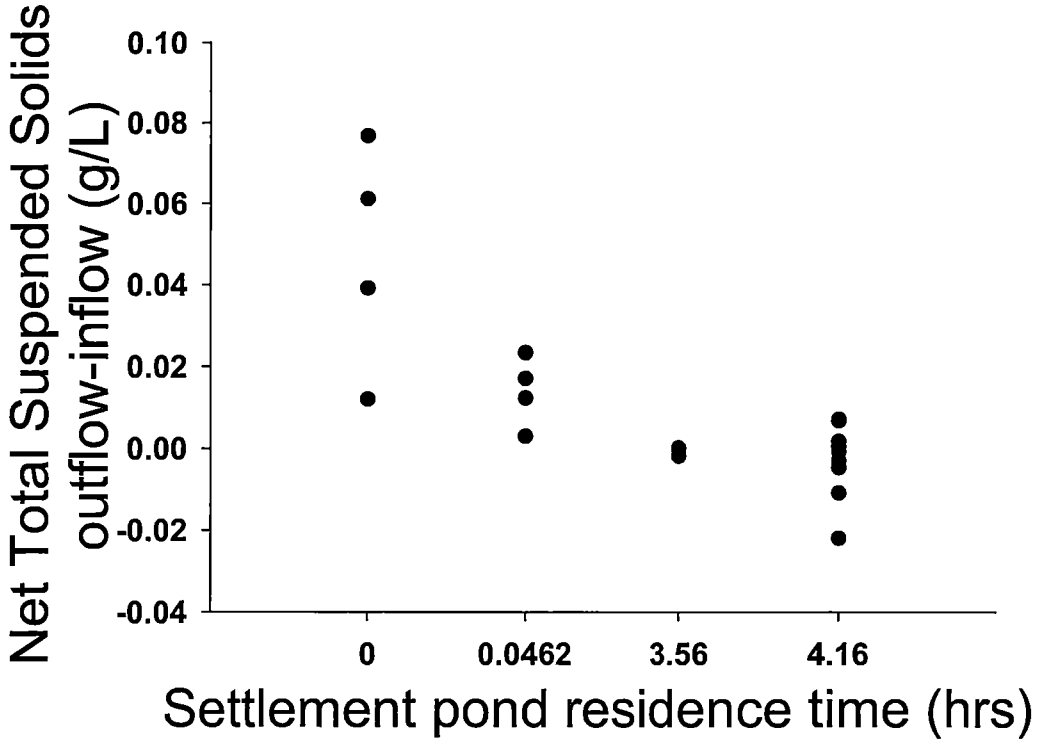

Figure 6.13: Sedimentation pond residence time versus the Total Suspended Solids (TSS) export (Net TSS value = farm outflow-farm inflow)

6.4 Discussion

6.4.1 Monitoring program

The results of the monitoring program indicate that export loads of all measured nutrients are generally below 2000g/day/farm and in the case of Farm C consumption of nutrients in the order of 1500g/day can occur. While these represent the two extremes of nutrient loads, there was a large amount of temporal variation as observed between the sampling periods, particularly with silicate and phosphate export loads. Dissolved nitrogen generally showed a trend of increasing loads for all farms.

These results combined with the monitoring information from Chapter 3 indicate that farms with greater biomass will export more nutrients into the marine environment than farms with lower biomass. Results of Chapter 3 indicated that at AFA nitrite, nitrate and phosphate (silicate always produced) were predominantly consumed when lower biomass was held on the farm (1.5 tonnes held on 31-8-02), and produced when greater biomass (18 tonnes held on 24-3-04) is held within the farm. This was particularly true for dissolved nitrogen which showed steady increases over the intensive monitoring program (Chapter 3). Similarly with the state-wide monitoring of Tasmanian farms, the operations with greater biomass (Farms A+B, i.e. above 10 tonnes approximately) consistently produced nutrients while the lower biomass farm (Farm C, below 10 tonnes) showed periods of dissolved inorganic nutrient consumption and production throughout the monitoring period.

Despite Farm B having almost double the abalone biomass of Farm A, the net export of dissolved nitrogen, silicate and phosphate between the two farms was similar. This indicates that while abalone biomass is an important factor influencing the production of dissolved nutrients there are other aspects (i.e. the farming system) that are likely to play an important role in determining the overall nutrient export. A possible reason for this difference in nutrient export may be the lack of sedimentation pond on Farm B. Chapter 1 showed that while AFA's sedimentation system did increase the concentration of dissolved nutrients into the water column (through the sedimentation of particulates which are remineralised into dissolved nutrients), in that case no particulates were exported into the marine environment. Therefore the absence of a sedimentation pond at Farm B may result in lower concentrations of dissolved nutrients to be measured

at the farm outflow, however certainly some amount of nutrients (particularly phosphate, and to a lesser extent nitrogen) will be exported from the farm in the particulate fraction (discussed below).

The advantage of sedimentation ponds in reducing the particulate load exported to the marine environment is shown by the three farms studied. Of all the farms monitored Farm B showed the greatest export of particulates to the marine environment with Farm A showing the next greatest average export while Farm C actually consumed particulates. Examination of residence times shows that an inverse relationship may exist between the residence time of the sedimentation pond and the concentration of solids exiting the farm outflow. Similar relationships between sedimentation pond residence times and solids retention have been found by other researchers (Henderson and Bromage, 1988; Toms et al., 1975) and based on this research and the studied farms, it can be seen that farms with sedimentation ponds are likely to have reduced or zero particulates exported into the marine environment. Farms without sedimentation ponds are likely to be contributing particulates into the marine environment and certainly these particulates (mainly consisting of uneaten feed and faeces) are likely to contain the nitrogen and phosphorus precursors for the production of dissolved nutrients such as ammonium and phosphate. Therefore, when monitoring farms which are likely to be exporting particulates (i.e. farms without sedimentation systems), the biochemical composition in terms of particulate N and P should be determined to give a total picture of the N and P export. This suggests that farms which have a proven ability to retain particulates through the implementation of a solids separation device may be able to monitor dissolved nutrients only.

Subsequently when comparing nutrients exported from a farm operating without a sedimentation pond to a farm operating with a sedimentation pond caution must be exercised. Even if total nutrient loads are determined (i.e. dissolved + particulate) and compared this may not be equivalent because dissolved and particulate nutrients have different effects upon the environment (Cheshuk et al., 2003; Islam, 2005; Schneider et al., 2005). Generally particulates have a more localised effect around the receiving waters where accumulation of particulates can cause increased BOD (Islam et al., 2004; Teichert-Coddington et al., 1999), smothering of marine life (Loch et al., 1996), decreased macrofauna abundance and diversity (Brooks and Mahnken, 2003) and require processing by micro-organisms before nutrients can be remineralised and assimilated into the environment (Boyd, 1992; Hargreaves, 1998). Dissolved nutrients on the other hand are readily assimilated into the marine environment and relative to particulates may be transported greater distances away from the discharge point (Islam, 2005) so that environmental impacts may be more dispersed. It is therefore possible for two hypothetical farms to have identical total nutrient loads, yet their impact upon the marine environment may be considerably different (assume identical receiving waters).

While there is some evidence in the data that each farm may have a unique relationship between DIN and P export load and feeding rate there is a significant relationship over all available data for three farms. Therefore in the absence of any other information the dissolved nitrogen loads can be predicted from feed rates and this is likely to be a useful guide for the planning and management of new farms (i.e. some state environmental departments in Australia require the prediction of the nitrogen loads from

abalone farms before the farm has been approved (States of New South Wales, South Australia, Victoria and Tasmania).

While N and P export loads can be predicted, some limitations exist surrounding the use of the information from this relationship. Firstly the relationship is known to be applicable to farms that have contributed data to the regression or have very similar farming systems. The wide variation in farming systems is likely to mean that each farm has a different capacity to produce and consume nutrients. For example, a farm in which cement tanks and drains are exposed to sunlight and have long residence times may actually have lower amounts of nutrients exported if benthic microalgae and macroalgae are more able to utilise the nutrients, proliferate and potentially be consumed by abalone. Conversely a farm feeding the same amounts as the above farm but using PVC pipes (i.e. generally shorter residence time and limited exposure to light) for growing abalone may not experience significant utilisation of nutrients within the farm and hence export more nutrients per unit feed. While these examples are hypothetical, and no farm monitored within the present study used PVC pipes as a grow out facility, they indicate the potential difficulties in extrapolating these relationships between N and P export and feed input to different farming systems.

Within the farms monitored there was a clear statistically significant relationship between feed rate and dissolved nitrogen/phosphorus export. However there is evidence that each farm has its own relationship for feed rate versus dissolved nitrogen export (Fig. 6.9). Therefore at different farms variable amounts of dissolved nitrogen may be exported for every kilogram of artificial diet fed to the abalone. The main causes of this difference are likely to be the presence/absence of sedimentation ponds (discussed above) and the

different diets (i.e. same company but different types of pellets) which are employed on each of the farms. Given that these different abalone diets are likely to have different formulations it is therefore possible that the nitrogen content also may differ. Unfortunately to protect the substantial investment of the feed companies (Fleming et al., 1996) into the formulation of their proprietary artificial diets, this information is not available. While the feed composition between diets may explain some of the variability in the feed input to dissolved nitrogen export relationship, it is likely most of the inter-farm variability results from the different capacities of the farms to retain the solid wastes (i.e. presence of SSD's).

The second limitation with this feed input to dissolved nitrogen export relationship is the accuracy of the relationship with the addition of more data points. Despite a increase in significance ($P = 0.018$ for AFA data alone, $P=0.001$ for combined farm data), the r^2 for the feed to dissolved nitrogen relationship using AFA data alone was 0.98, yet with the addition of the other 2 farms (i.e. 8 more data points) the r^2 was reduced by 28% to 0.708. This is a substantial reduction in the accuracy of the prediction of nitrogen export and this variation may be reduced if individual relationships were developed for individual farms. A trade off exists between the sampling effort devoted to each farm and the ability to characterise abalone farming as a whole. However, given that there is insufficient data to get a statistically significant relationship for each farm the overall equation provides a useful management tool to make an initial estimate of dissolved N export for operations with similar farming styles and a sedimentation pond

Abalone biomass may also be used as a tool to predict the export of dissolved nitrogen from abalone farms. The biomass-export relationship demonstrated here appears

to be more precise than the feed-export relationship and thus offers a more robust means of estimating dissolved nitrogen export, however, the difficulties and effort required to measure on-farm abalone biomass are certainly greater. Biomass estimates commonly require a representative sample (could be thousands of animals) to be taken and measured for weight (or length) which is a time consuming process relative to feed monitoring (a process which takes minutes to complete). Therefore even though biomass is a more reliable variable for the prediction of dissolved nitrogen export, the frequency of biomass measurements on farms may limit its use as a tool for resource managers interested in knowing the likely environmental impact of a particular farm at a particular time. It does, however, provide a useful tool for the assessment of the likely discharge load from any new proposal where the target production biomass is stipulated.

Both daily feed rate and farm biomass may also be used to predict the phosphate export from the farm outflow. While both variables may be employed, feed rate is probably more directly related to export load given the findings of chapter 1 with respect to P leaching from formulated feed. The main source of variation in the relationship between feed rate and dissolved phosphate in the water is likely to be the type of diet. While all diets are derived from the same manufacturer, they are different in pellet sizes and formulation which is likely to cause different rates of leaching (Fleming et al., 1996; Guzmán and Viana, 1998). Another factor which may be causing some variation in the observed feed-export relationship for P is the hourly fluctuations in the phosphate concentrations which are characterised by a short, sharp peak following the formulated feed entering the water. Chapter 2.3 shows phosphate concentrations can vary by a three fold factor (1.8μm to 0.6μm) over 24 hours with increases in concentration of up to 1μm

over 10 hours. Assuming Farms A and C show similar diurnal patterns of phosphate concentration it is likely that feeding and sampling times will have a large affect upon the relationship between DIP and feeding rate. Typically sampling for the statewide monitoring program occurred between 0800-1300 and therefore was unlikely to be subject to the same wide fluctuations in P exhibited over 24 hours as shown in chapter 2 (assume relatively consistent feeding times). Consequently the feeding and/or sampling regime may be a source of some variability in estimating phosphate loads however there is unlikely to be the three fold variation as seen at AFA over 24 hours.

From the results it can be seen that there are strong relationships between the feed input and abalone biomass with dissolved phosphate and dissolved nitrogen exported into the marine environment. However the diverse nature of the abalone industry has seen many different farming styles proliferate (Fleming and Hone, 1996). The different farming styles, coupled with different tank and farm residence times, ambient water temperatures, sedimentation pond, species cultured and farm practices are all likely to cause some variability in the relationship between dissolved nutrient export and feed rate or biomass. Ideally each farm should have their own regression relationship, however this may not be completely practical given the sampling effort required to achieve this. Nevertheless, the relationships demonstrated here provide us with some basic tools useful for the management of abalone farming and will help to define where it is likely to sit within the spectrum of marine resource users who input nutrients into our coastal waterways.

6.4.2 Management

Within the field of environmental management, difficulties arise between a mismatch of what is ideal for the long term sustainable use of the marine environment (i.e. hypothetical complete understanding of the all environmental impacts of anthropogenic disturbance within a given area), and what is practically and economically feasible. For this reason the effects of many industries effluents on the marine environment have not been completely studied despite the proliferation of any given industry. We are now in a situation where land based sources account for 77% of all marine pollution (Williams, 1996). This includes export from rivers as well as direct discharge into the ocean. Hence there is a need to understand the relative contributions of every marine resource user from sewage outfalls to agricultural runoff to abalone farming. Each industry should be striving to characterise their own inputs into the ocean, and gain a perspective of where they sit within the spectrum of marine users. Once this picture is established, broad scale management is likely to be a great deal more effective and structured (discussed further below).

Until recently the environmental impacts of abalone aquaculture had not been studied despite there being numerous farms around the country in full operation. This had caused some pressure to find answers and to the credit of the Abalone Farms Australia, Tasmania, the effects of abalone farming upon the marine environment have now been extensively studied. Potentially some of these findings may be applicable to form the basis of a state-wide abalone aquaculture operational policy, however, it is not known how applicable the environmental impact assessment of one abalone farm is to another as

there are simply too many site specific environmental variables involved. With limited resources and a large number of possible environmental impacts this thesis only addresses a few of the main issues. Despite this it may be broadly concluded that the excess effluent nutrients are likely to cause the growth of some algae species and seemingly this is likely to be the foremost of concerns if a similar biomass scale close to AFA's is maintained.

The environmental management of land based abalone farms is governed by local councils in the state of Tasmania. Consequently different environmental standards are often required and each individual farm is governed on a case-by-case basis. Typically, the environmental focus has been on the nutrient export to the marine waters and deciding what is an acceptable level of nutrient export for a given area has caused much debate (Boyd, 2003). Local planning agencies require a suite of research (i.e. prediction of nutrient loads and environmental impacts, relationships between local conditions and environmental impacts, development of protocols for minimising impacts) with which to access and further use to assess risks and make informed decisions about abalone aquaculture developments in their local shire. This will allow effective planning and minimise the likelihood of adverse effects of an abalone farm on a local environment. Further in order to assess the impact of abalone farming relative to other users of the marine resource the data from AFA and the other farms monitored should be assumed to be representative of all abalone farms in the State (until proven otherwise).

With the findings of this study, information as to the likely nutrient export and likely effect on the marine environment may be applied to other farms, hence providing some of the tools needed to form the basis of these type of planning decisions. However,

in addition to the likely nutrient export and environmental impact, the assimilative and dilution capacity of the receiving waters are of great importance in determining the ecological impact of a given discharge. While these days the concept of assimilative capacity is deemed obsolete as a management tool (replaced by precautionary principle (Williams, 1996)), it is worth mentioning given the diverse nature of abalone farm discharge environments. Clearly a deep, high physical energy coastline would be more likely to dilute effluent than a sheltered shallow bay. Similarly, a sandy beach is likely to have a greater assimilative capacity than a shallow rocky outcrop with its limited surface area. The broad range of environments into which abalone farms may discharge suggests that management strategies need to be flexible and that narrow criteria should not be broadly applied across all farms. It should be stated that the likely impact of most farms in the 20 tonnes biomass range is likely to be proliferation of dissolved nutrient scavengers. Our results showed a diverse range of nutrient concentrations for the farms despite similar farming systems. The main factor causing the variation was biomass. Subsequently management strategies may need to incorporate biomass when assessing risks of farms on the marine environment.

Another major consideration is the use of the marine resources amongst the community and other resource users (Fernandes et al., 2001). In particular the suite of other users exporting nutrients to the marine environment should also be examined to determine what relative level of risk a single operation represents as well as the cumulative effect of all operations in an area (often difficult due to lack of information) (Ackefors and Enell, 1990). This study has provided information as to the relative environmental risks and nutrient export of abalone farms in Tasmania. This allows

planning authorities to make informed decisions to ensure the appropriate level of regulation is applied to the appropriate potential risks. It is now up to other industries to follow and provide evidence of their impact upon the marine environment.

Around Australia there are a number of different operations that input nutrients into inland, estuarine and marine waterways. Table 6.2 shows a number of different comparable operations within Tasmania and around Australia exporting effluent into the freshwater and marine environments.

Table 6.2. Various operations within Tasmania and around Australia exporting effluent into the freshwater and marine environments

	Output	N approx annual load (tonnes)	P approx annual load (kg)	TSS approx annual load	Discharge Environment	Reference
AFA	20 tonnes of abalone	0.11-0.39	25-40	None	Semi exposed coastline	
Tas Alkaloids		0.140	14,000	Not stated	Inland river	(Koehnken, 2001)
[a] **Prawn Farm A**	44.2 tonnes of prawns	4.9-13.6	Approx 618	446.15 tonnes	8km from river mouth	(Jackson et al., 2004)
[a] **Prawn Farm B**	310.2 tonnes of prawns	16.5-26.7	Approx 618	596 tonnes	Mangrove creek	(Jackson et al., 2004)
[a] **Prawn Farm C**	41.9 tonnes of prawns	2.9-4.7	Approx 618	13.7 tonnes	River/estuarine	(Jackson et al., 2004)
[b] **St Helens (Tas) sewage**	Pop = approx 800 permanent, 8000 during summer	4.8-6.1	2300-3300	27.7-40.5 tonnes	Estuarine	(Koehnken, 2001)
[b] **St Marys (Tas) sewage**	Pop. = approx.600 people	1.5-1.8	530-730	6.2-18 tonnes	Inland river	(Koehnken, 2001)
Salmon farming in Huon estuary	1997 output= 5000 tonnes	137	Not stated	Not stated	Estuarine	(CSIRO, 2000)
Oyster and shellfish	Organic enrichment and reduction in carrying capacity. Risk = Low				Estuarine	(Crawford, 2003b)
[b] **DPIWE Emission limits for sewage treatment**	500,000L/day treatment plant	1.3-1.8 tonnes	182-547	2.7-3.7 tonnes	Marine	(Koehnken, 2001)
Intensive agriculture	[c] Average Tasmania farm size = 444.9 ha	50 kg/ha/yr = [d] 11.1 tonnes/yr	20 kg/ha/yr = [d] 4.5 tonnes/yr	Not stated	Inland rivers/estuarine	(Harris, 1994)
Vegetable processing plant Ulverstone, Tas	5,000,000L/day flows untreated	High BOD and 30°C water have been recorded at discharge			Inland river	(Koehnken, 2001)

[a] Total N and P figures based on initial and final loads/kg/ha/day for the production cycle

[b] Total N and P figures based upon 50%ile and 90%ile results multiplied by maximum permitted flow (kL/day).

[c] From Austats "Agricultural establishments and area of holding, Tasmanian regions(a), Australian Bureau of Statistics, 2001

[d] based on 50% usage of total farm size for intensive agriculture

Table 6.3: Comparisons of reported effluent characteristics contributed into waterways by users around Tasmania

	Effect	Reference
Dairy sheds	N+P and solids	(Gutteridge, 1996)
Cattle and sheep farming	Solids, bacteria	(Bobbi et al., 1999)
Forestry	Solids, pesticides, Water usage,	(Koehnken, 2001)
Stormwater runoff	Solids, nutrients chemicals	(Koehnken, 2001)
Agriculture	Pesticides, nutrient, solids	(Koehnken, 2001)
Cheese factory	BOD	(Koehnken, 2001)
Abattoirs	COD, BOD, nutrients	(Koehnken, 2001)
Mining	Metals, sulfate	(Koehnken, 2001)

It can be seen that there are numerous users of the marine environment who input nutrients and chemicals into waterways and ultimately the ocean (Table 6.2 and 6.3). As mentioned some of these users input nutrients through a point source (i.e. abalone farm,

prawn farm) while other users input through diffuse sources (i.e. agricultural runoff). Generally diffuse sources of nutrients tend to be quite difficult to quantify due to high variability both temporally and spatially (Harris, 1994). Nevertheless diffuse sources such as agriculture represent a large source of nutrients and particulates that are input into waterways and in many cases are likely to end up in the oceans. Monitoring point source outflows is relatively simple and more cost effective and therefore regulation through monitoring programs is easily achievable. It is primarily for this reason that monitoring programs and regulation has proliferated for point source outputs while diffusive sources such as agriculture have been allowed to operate for many years with little or no water quality monitoring(Council, 1996) . This situation is changing and within Tasmania and worldwide today there is a great deal of research into management strategies that reduce agricultural runoff and dairy effluent into waterways (Bobbi et al., 1999; Bowman, 1999; Cotching, 2000; Koehnken, 2001)

Table 6.2 provides us with a list of the different industries which input nutrients and particulates into the marine environment (some indirectly through freshwater). The addition of nutrients and particulates has led to many documented cases of algal blooms (Bowling and Baker, 1996; Harris, 1994; Wee et al., 1992) (both harmful and more benign) and degradation of the riverine systems (Patterson and Watts, 2003; Prosser et al., 2001). While not all the waste input into freshwaters will end up in the marine environment in dissolved form, many studies suggest a large proportion is likely to be exported into coastal waters (Dagg et al., 2004; Justic et al., 1995; Lourey et al., 2001). Therefore we must consider the effects of riverine input when considering marine coastal management as each river is a collective 'point source' of many users. From the results of

Table 6.2 we can see that across the spectrum of users an abalone farm producing 20 tonnes of animals exports approximately 0.4 tonnes of nitrogen and 40kg of P. Comparatively, an average sized Tasmanian intensive agriculture farm produces approximately 11 tonnes of nitrogen and 4.5 tonnes of P. Tas alkaloids is estimated to produce approximately 14 tonnes of P discharging into an inland river system (i.e. a phosphate limited ecosystem). Total abalone farming around the state of Tasmania (i.e. the 8 farms) may reach the rough equivalent to a sewage treatment plant which services the town of St Marys (i.e. pop of 600 people). However this alone is not to say that abalone farming is not a potential risk to the marine environment as the industry in Tasmania is currently in its infancy and only produces approximately 100 tonnes. A survey of various Tasmanian farms indicates a desire to be producing at least 70 tonnes each (Ho et al., 2004). Therefore Tasmanian abalone production may increase by four to five fold in the forthcoming years which would then have the same nutrient input as a single average sized, intensive Tasmanian agriculture farm. Using this method of comparison, Tasmanian abalone farming is likely to be relatively benign when you consider the spectrum of other users who input nutrients into the marine environment. However despite these comparisons, the location of abalone farms does need to be considered as within Australia approximately 85% of the population resides within 50km of the ocean(Austats, 2001). Further to that in Tasmania 99% of the population resides within 50km of the ocean (Austats, 2001). Hence if policy makers were to base planning decisions on nutrient export alone, it would seem that abalone farming is at the lower end of the 'polluter' spectrum; however, the coastal location certainly adds some level of increased social sensitivity amongst communities. Despite this the argument (made by

environmental groups and general community – Ho, Personal observation, 2002-2005) that abalone farms are severely polluting marine environments seems unfounded. Nevertheless, while these results provide direction as to where attention should be focussed, we must remember that all users of marine and freshwater resources have the collective responsibility of reducing and minimising their environmental impact

Another approach that policy makers may like to consider is the relative efficiency of culturing abalone. One means of doing this is to measure the nutrient export for a given amount of abalone production. Comparisons of nitrogen exported per tonne of product shows that Abalone Farms Australia is more efficient than prawn farming (between 0.053-0.308 tonnes-N exported/tonne-prawn produced) (Jackson et al., 2004) and salmon farming (approx. 0.27 tonnes-N exported/tonne-salmon produced -(CSIRO, 2000)) exporting between 0.0054-0.0195 tonnes of nitrogen for every tonne of abalone produced. However abalone farming is likely to export much more nitrogen than cultivation of shellfish such as oysters and mussels.

Overall it would appear that the farming style and husbandry practices of Abalone Farms Australia causes them to sit at the lower end of the spectrum of the nutrient (N and P) exporters into the marine environment. With respect to other abalone farms around Tasmania, it is likely that they also will be contributing relatively low levels of N+P into the marine environment. This can be shown through the strength of the statistical relationships between feed input or abalone biomass and N or P exported (i.e. highly significant relationships). It should be stressed, however, that these comparisons of industries discharging into waterways are based solely on N, P and TSS loadings. Table 6.3 shows qualitative data for other users who input effluent into waterways. It can be

seen that nutrients are just one component of the suite of potential threats to the environment and ideally a spectrum relative to other industries should be developed for each of the different components of an industry's effluent (i.e. BOD, particulates etc). These could then be subjected to a risk assessment as a kg of dissolved nutrients may be much less of a concern than a kg of pesticides.

6.5 Conclusions

Of the forms of Australian aquaculture for which information on waste discharge was obtained, abalone farming was shown to be a relatively clean industry which exported low amounts of N+P into the environment especially per unit biomass of production.

Abalone farming, like any other industry, requires some level of regulation. Relative to the spectrum of nutrient inputs to the marine environment from other industries the current output from abalone farming does not represent a significant threat to the marine environment of Tasmania. Sedimentation ponds reduce the export of particulate matter, reducing the overall nutrient load but increase the fraction released as dissolved nutrients. Care in locating farms and their effluent discharges combined with good farm husbandry should make it possible for farms to have relatively little impact even at a local scale. In the future abalone farming may pose an increased risk to the marine environment if production levels are increased significantly.

6.6 Recommendations

Future management of abalone farms may employ trigger levels of production or effluent loads as an indicator of situation which might require more intense monitoring. At present there are many abalone monitoring programs around Australia; however, differences between sampling procedures are a likely source of variability making comparisons between data sets unreliable. Development of a standard protocol for taking nutrient samples and analysing them would improve compatibility making comparisons more valid and would be a more efficient use of resources. Monitoring programs should consider including more rigorous information collection to allow total loads to be calculated and further a relationship between feed rate and effluent nutrients developed for each farm. Information as to the state of the farm (i.e. cleaning or no cleaning occuring) when collecting samples and the presence/absence of solids settling devices should all be documented. As well as this the development of a step by step set of protocols outlining the most appropriate collection times (around midday), sample collection locations and methods, sample storage and sample transport to the laboratory. Only after this information is collected then a better perspective of the abalone industry can be gained and ultimately effluent nutrients may be predicted from feed rates (i.e. water quality sampling may be eliminated if the feed/nutrient relationships are strongly significant).

Chapter 7: General Discussion

7.1 General Discussion

The declining worldwide wild catch of abalone (Gordon and Cook, 2001; Hone and Maguire, 1996), combined with the high demand for abalone and seafood by Japan, China and USA (the world's largest abalone consumers) (Oakes and Ponte, 1996) has spurred the increase in abalone aquaculture both within Australia and around the world (Hone and Maguire, 1996). At a time when there is increased competition for resources on the Australian coastline and oceans, all users are facing increased requirements by regulators to quantify their environmental effects/impacts and ensure long term environmentally sustainable practices are in place. Consequently there is a need for research to provide a perspective on not only the present and likely future environmental implications associated with abalone aquaculture operations but also their potential risks. Appropriate research allows for informed management decisions, development of sound policy; and suitable and sustainable allocation of marine resources to users.

The results of studying numerous farms around Tasmania indicate that the practice of culturing abalone in land based facilities is likely to export nutrients into the marine environment. The nutrients of primary concern are nitrogen and phosphorus based nutrients; namely ammonium and phosphate. While the original source of these nutrients is from the addition of formulated feed into the culture tanks, the component of leaching directly from the feed into the water column is likely to be negligible for ammonium and significant for phosphate (up to 30% within 2 hours). Future work to improve the binding of P in the formulated feeds may help to overcome the loss of this nutrient through leaching. The particulate waste generated in the abalone culture tanks is primarily

composed of uneaten feed and faeces and is a rich source of nutrients and labile organic material. Under normal practice this material is periodically flushed from the abalone culture tanks and into the drains and sedimentation ponds (if present) where it is broken down by a host of organisms including bacteria, polycheate worms, and Malacostraca crustaceans. The action of these organisms within the sediments of a sedimentation pond causes the remineralisation of particulate waste constituents/nutrients into the water column where utilization by chemoautotrophs is likely to occur. These processes of remineralisation and utilization are likely to be strongly influenced by both environmental conditions within and outside of the sedimentation ponds. The seasonal and diurnal variation in water column nutrient concentrations is likely to be driven by primary production and where solar irradiance and daylength, water temperature and the dynamics of the biota inhabiting the sedimentation ponds may play a role. If no sedimentation ponds are present then it is likely the above processes will occur in the local marine area surrounding the end-of-pipe, however shoreline wave energy may also be a factor affecting breakdown and remineralisation.

The total loads of dissolved nitrogen and phosphate can be predicted from the amount of formulated feed input into the farm waters. This relationship was shown to apply to AFA and other farms around the state of Tasmania. Such a relationship is likely to be of particular use to industry and regulatory authorities as feed rates are a commonly monitored and easily reported parameter. Individual feed to nutrient relationships may be more appropriate as variation between the farms in terms of husbandry operations, environmental conditions and differences in infrastructure are likely to cause some inconsistency in the observed relationship. An improved or less variable relationship was

found between abalone biomass and nutrient export from the farm, such that a much greater accuracy in the prediction of the dissolved nutrients exported from the farm was observed, particularly when multiple farms were combined. Despite this, the application of the biomass to nutrient export relationship may not be as useful as the feed rate to nutrient export relationship to either regulatory authorities or farmers due to the difficulty and low frequency with which the majority of farms monitor abalone biomass.

The diurnal flux in nutrient export indicated that over a 24 hour period ammonium concentrations varied by two orders of magnitude and phosphate by up to three orders. This has implications for the interpretation of the monitoring program results and indicates that time of sampling is an important variable in accurately quantifying nutrient export and potential environmental impact. Therefore until diurnal variations are characterised over numerous conditions, consistency in the time of sampling between sampling periods is of crucial importance for meaningful data from monitoring programs.

The export of particulates into the marine environment from abalone farms was observed to be controllable through the use of solid separation devices. The use of solid separation devices is likely to reduce the environmental impacts of abalone farming given that particulates are often associated with increased Biochemical Oxygen Demand (Islam et al., 2004; Michael Jr, 2003; Teichert-Coddington et al., 1999), blanketing of marine life with organic matter (Loch et al., 1996) and increased nitrogen and phosphate enrichment of the marine environment (Crawford, 2003a; Porrello et al., 2003). In comparison dissolved nutrients are more easily dispersed by wave action and local currents and are quickly assimilated into the marine environment (Crawford, 2004). The

sedimentation pond system at AFA proved adequate for the removal of suspended solids from the effluent waters with only one case out of 10 when net particulates were exported from the farm. This occurred during tank construction when terrestrial matter was washed into the drains and sedimentation pond during a heavy rainfall event. These particulates were not efficiently removed by the sedimentation ponds due to the halocline which formed and the particulates exited the farm untreated as the sedimentation pond water exits from the surface of the pond on its way back to the ocean. Other farms which were monitored around the state of Tasmania showed that particulates were not always retained within the farm but rather exported to the marine environment. Farms with a small or no sedimentation pond were more likely to export particulates than farms which had larger sedimentation ponds with longer residence times. Future work may examine the solid separation device efficiencies and/or appropriate residence times of sedimentation ponds for farms with different biomass and water usages.

The export of nutrients from AFA caused community shifts within the intertidal region surrounding the farm outflow. During late spring to early summer the proliferation of the nutrient scavenging seaweeds such as *Ulva* and *Porphyra* caused complete coverage of some rock surfaces near the outfall. *Porphyra* extended predominantly in a southerly direction to approximately 50m from the end of the discharge pipe, however the exact contribution of the farm effluent to the *Porphyra* abundance outside of the sampled quadrats is unknown as *Porphyra* abundance also increased at the control sites over the same period. Evidence from the plume study suggests that in the intertidal region beyond 50m to the north and south between 3.7 and 7.5%, respectively, of the end-of-pipe effluent concentrations remained. Consequently it is plausible that there is likely to be

minimal effect of the abalone farm on the macrophyte community past 50m. Despite the proliferation of *Porphyra* sp. during late spring/early summer the seaweed abundance in the intertidal region declined during the late summer periods, likely due to desiccation. Although not conclusive there were indicators of increased grazers at the impact site relative to the control sites. There was no statistically significant effect of AFA discharge on the filter feeding community of the intertidal community in the vicinity of the outfall.

This study demonstrated that the annual loads of nutrient export from AFA are likely to be low relative to other users who are contributing nutrients to the marine environment. There is however a responsibility of all marine resource users to not only understand their impact upon the marine environment, but also to reduce their effect on the marine environment. As demonstrated in this study, the growth of seaweed on rafts within the abalone culture tanks is not only capable of reducing effluent ammonium concentrations by up to 71% (daily average = 48%), but also provides a shading mechanism and also a nutritious food source capable of supporting abalone growth equivalent to that of the formulated feed (Boarder and Shpigel, 2001).

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Appendix

Calculation per litre of seawater flowing:

Tank Silicate Conc = 217-340 µmol/L
Tank volume = 10,000L
Farm daily flow rate 8,640,000L
Trial duration = 3 days

Si in 1 tank = 217-340 µmol/L x 10,000L = 2,170,000-3,400,000µM
Water flow over 3 days = 8,640,000L x 3 days = 25,920,000

Ability of empty to tank to raise Si conc. of water column =

2,170,000/ 25,920,000 = 0.084µmol/L of ambient seawater

3,400,000/ 25,920,000 = 0.131µmol/L of ambient seawater

One empty tank has the capacity to raise effluent nutrient concentration by 0.084 - 0.131µmol/L ambient seawater

Calculation per square metre of tank surface

Tank Silicate Conc = 217-340 µmol/L)
Tank dimensions (6.5m x 2.5m) (1.2m - 1.6m depth)
Tank Surface area = 41.45m^2
Tank Volume 10,000L

Moles of Si in one tank

217-340µmol/L x 10,000L= 2,170,000 µM

µM/m^2 = 2,170,000/41.45 = 52,352.23µM/m^2

mM/m^2 = 52.35

Hence over 3 days each square metre of concrete within the tank leached 52.35mM of Si

Artificial diet silicate content

Artificial diet contains 130ppm Si
Average daily feed rate over period sampled = 30.88Kg
Daily flow rate = 8,640,000L

Amount of Si in daily feed = 130ppm x 30.88kg = 4014.4mg

4014.4mg/ 28.08 (Si molecular wt) = 142.96 μM Si in daily feed

142.96 mM = 142,962.96μM

μM/L ambient water = 142,962.96 / 8,640,000 = 0.0165μmol/L

i.e. the artificial diet has the capacity to increase every litre of ambient water by on average 0.0165μmol/L

The above calculations assume even leaching over the periods sampled.

Calculation of Margalef's diversity index
$D_{Mg} = (S - 1) / \ln N$

S = number of species recorded
N = the total number of individuals summed over all S species